UNIVERSITY OF LONDON THESIS

Degree phd Year 2007 Name of Author JULIE ANN EKLUND

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20 December, 2006

The Effects of Preparation and Conservation Treatments on DNA

A thesis submitted for the degree of

Ph.D. in Archaeology (Conservation)

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Abstract

Both human and animal remains are held in a variety of museum collections including, but not limited to: art, archaeological, anthropological, ethnographic, biological, zoological, palaeontological, geological, anatomical and medical collections. Little is known about the effects of chemical preparation and conservation treatments, administered either in the field or in the museum, on DNA in skeletal and soft tissues. Treatments administered within different disciplines are known to vary, but little comparative research has been carried out to date. A literature review was undertaken to document and compare preparation and conservation approaches within these different fields. A database was compiled of published past treatments used for the following purposes: acid preparation, adhesive, adhesive for spirit collections, barrier coat, bleaching agent, chelating agent, cleaning agent, consolidant, degreasing agent, dry soft tissue preservative, drying agent, finishing material, fungicide, moulding/casting material, packing material, pesticide, photographic aid, sealant, skeleton preparation, solvent, and wet soft tissue preservative. Some of the most commonly and best documented of these materials were then used to assess their effects on DNA by treating DNA fragments of known length *in vitro*. A case study was also carried out on ancient and recent Egyptian animal mummies to assess the effects of the mummification process on DNA. It was found that the majority of treatments tested in this study were damaging to DNA, but a few, primarily organic solvents, were not. Basic mummification consisting of an ethanol wash and desiccation using natron was also found neither to be damaging to DNA in the short-term nor to inhibit amplification by PCR. The results of this research will be useful both in determining collection materials likely to be more or less suitable for DNA analysis and in suggesting preparation and conservation materials and methods suitable for DNA preservation.

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Preface

As an archaeological conservator with a background in field archaeology and human osteology, I regularly work interchangeably as a conservator, archaeologist and osteologist. This has led to an interest in promoting interdisciplinary approaches to conserving archaeological materials, as occasionally the aims of the conservator may be at odds with the research objectives of archaeologists. Recently, however, it has come to my attention that researchers from other disciplines intent on using collection materials are often completely unaware of the role of conservators, as well as the methods and materials used to prepare and preserve museum collections and the potential effects this may have on their results. Similarly, conservators are generally unaware of the material requirements for many research methods which may be used to study specimens and objects in museum collections. Furthermore, when considering requests for samples, many collection managers may not be cognisant of the conservation history of objects which may be incompatible with the material requirements for certain research methods. With the increase in biochemical research, using museum collection material, this issue is of increasing importance.

This project was approached from a conservation point of view, with the hope of promoting interdisciplinary collaboration and communication regarding the potential effects that conservation treatments may have on research using archaeological and museum specimens in general, and with specific reference to DNA analysis. However, this is not intended to be a biochemistry thesis. Biochemical methods were merely used as a research tool to answer a number of conservation questions. The results are relevant, however, to biochemists as well as conservators, curators, museum managers, archaeologists, biologists, students and other researchers, as better knowledge about the effects of conservation treatments on DNA may shed light on previously published data or sampled specimens and should affect future excavation and collection care strategies.

Throughout this thesis, both human and animal remains are collectively referred to as "specimens" or "collection material". I am aware of the ethical considerations surrounding the treatment of human remains as well as the respect and sensitivity due to the memory of the individuals whose remains may be held within a collection as well as the descendants of those individuals. I am also aware of the implied objectification associated with this choice of words. I have chosen this wording simply as a matter of brevity, and in no way intend any offence.

Acknowledgements

Many thanks are due to my supervisors, Prof Simon Hillson, Prof Clifford Price and Dr Ian Barnes for their support, encouragement and tolerance throughout this venture. I am deeply indebted to The Centre for Genetic Anthropology, particularly Dr Mark Thomas, Abi Jones and Beth Caldwell for use of facilities, training and valuable insight.

Ancient Egyptian animal mummy samples were made possible by the kind permission of Richard Sabin. A recent rabbit mummy tail was graciously sacrificed by Dr. Salima Ikram.

Materials and supplies were generously supplied out of the goodwill of a number of kind people, most notably Sandra Bond and James Hales.

Thanks are also due to the many archaeology, conservation and museum professionals who have offered their knowledge and expertise, including: David Pinniger, Julian Carter, Adrian Doyle, Kathy Tubb, Dean Sully, Martyn Cooke, Gretchen Voeks, Melanie Pereira, Jessie Johnson, Margot Wright, Ian Carroll, Helen Chatterjee, Jo Hatton and Nancy Odegaard.

This research was funded by the Overseas Research Students Awards Scheme and the Institute of Archaeology.

Many thanks are also due to Prof Peter Ucko, who is in many ways responsible for putting me in the position to embark on doctoral research.

Most importantly I must thank my husband, Daegal Tsang, for listening to my incessant rambling on about this research, collecting me late at night from the lab, and generally putting up with this project for the better part of the last five years!

Chapter 1. Introduction

1.1. Premise

Both human and animal remains are held in a variety of collection types, including but not limited to: art, archaeological, anthropological, ethnographic, biological, zoological, palaeontological, geological, anatomical and medical collections. Such collections exist for the purposes of teaching, reference, research or exhibition. Treatments administered within different collections for both specimen preparation and maintenance are known to vary, but their effects on deoxyribonucleic acid (DNA) are still not well understood. Although there is an increasing tendency away from chemical use on collection materials within museums, a wide variety of chemicals have been employed in the past for processing skeletal and soft tissue material, pest control, exhibition preparation of specimens and other purposes. Such treatments potentially could have profound effects on the biochemistry of the specimen. Conservators are beginning to appreciate the potential effects their actions may have on the future utility of specimens in collections for biochemical analysis and to seek methods for preserving the molecular integrity of material in their care. The objective of this study is to be a conservation driven investigation of the effects on DNA of several of the most commonly documented chemicals in preparation and conservation treatments administered to human and animal remains. This study should both educate biochemists of the potential effects a specimen's treatment history may have on research results and engage the conservation profession in the preservation of biomolecules.

1.2. Background

Broadly defined, conservation is the discipline dedicated to the preservation of our cultural and natural heritage. The field of conservation evolved from a long-standing craft trade, through a series of developments in the first half of the 20th century to emerge as a profession in the 1950s with the creation of the International Institute for Conservation (Clavir 1998; Sease 1998: 749). In order to differentiate themselves from other practitioners, conservators adopted various principles of conservation, which were outlined in codes of ethics and codes of practice adopted by the different national and international professional conservation associations (Sease 1998).

These codes share many similar principles of conservation which are of relevance to this study. The primary aim of conservators is to prevent damage and deterioration to materials in their care, "governed by an informed respect for the integrity of the property, including physical, conceptual, historical and aesthetic considerations" (Canadian Association for Conservation of Cultural Property and the Canadian Association of Professional Conservators 2000: 1) through minimal intervention, and selecting materials and methods that will have the least adverse

effect. Conservators should strive to maintain materials "as nearly as possible in an unchanging state" (ICOM Committee for Conservation 1984). The consequences of any methods or materials used "should not interfere, if at all possible, with any future examination, treatment or analysis" (European Confederation of Conservator-Restorers Organisations 2002). Preventive conservation measures, or indirect actions aimed at limiting damage such as maintaining environmental conditions conducive to material preservation, should be taken into account prior to any physical intervention. Furthermore, conservators must cooperate with other scholars to distinguish between "intervention that enhances the qualities of the object and that which is detrimental to its integrity" (ICOM Committee for Conservation 1984).

Although preserving the genetic integrity of collection materials is not specifically advocated in these conservation codes of practice, many of the sentiments expressed are supportive of this cause. Minimising change and damage to specimens, only using materials and methods that will not adversely affect future analyses, as well as working with other researchers to identify the best means to preserve materials are all measures that could promote and enhance the preservation of biomolecules in collection materials. Currently, there is little guidance available for conservators to proactively preserve DNA in collection materials.

In the past, the conservation of bone and soft tissue both on site and in collections has largely been guided by the desire to preserve morphology as a response to prevailing research methods (Horie 1992). Research on osteological collections was based on visual inspection and measurement, making morphological preservation paramount. Soft tissue required chemical intervention to avoid putrefaction, and many methods to preserve and maintain such materials in either a wet or dry state have been invented. The idea that inadequate or misguided conservation treatment could be the source of major damage to collections material was convincingly argued by Stephen Williams in his PhD dissertation, *Destructive Preservation: a Review of the Effect of Standard Preservation Practices on the Future Use of Natural History Collections*, which concludes that "because "destructive preservation" is directly in conflict with philosophies and practices of modern conservation, most of the preservation activities associated with natural history collections should never be confused with conservation until major changes are made" (1999: 161). Many of the problems identified by Williams are not unique to natural history collections.

Currently, there is a revived interest in human and animal hard and soft tissues in various collection types, and much of this interest is associated with increasing biochemical research utilising DNA. DNA is a complex polymer consisting of two strands of an alternating phosphate and 5-carbon ring sugar backbone connected by pairs of bases (basepairs or bp) to form a double helix (Figure 1). The two backbones of the double helix are joined by pairs of

bases in a predictable manner; guanine (G) bonds to cytosine (C) and adenine (A) bonds to thymine (T). There are some three billion nucleotides in the human genome, and it is the sequence of bases on each strand that contains and passes on genetic information, and which is of interest to biochemists.

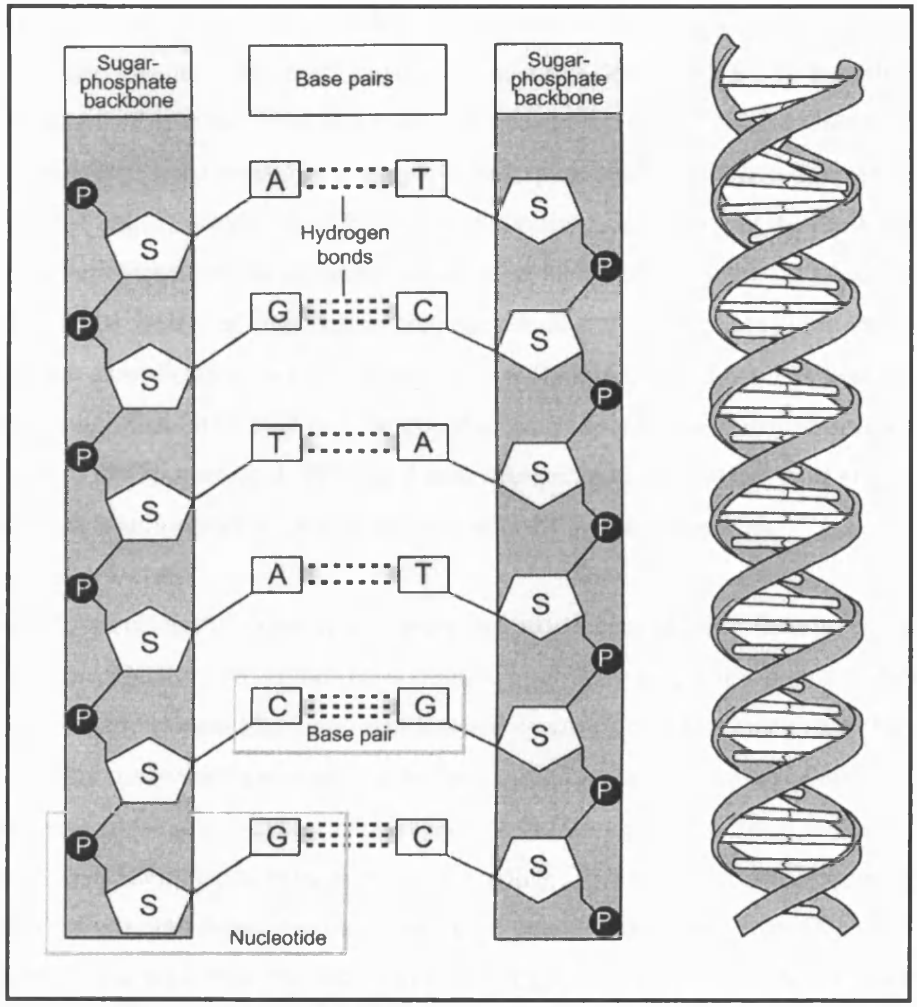


Figure 1. Diagram showing chemical groups that form DNA and how they are configured in a double helix (National Human Genome Research Institute 1994-2006).

The DNA of living organisms is susceptible to damage by a variety of mechanisms. During life, repair mechanisms attend to damaged sites, but after death DNA damage accumulates. Ancient DNA is primarily thought to be affected by hydrolysis and oxidation (Lindahl 1993). Unless inactivated by conditions such as rapid desiccation, low temperatures or high salt concentrations, endogenous nucleases begin to degrade DNA when an organism dies (Hofreiter et al. 2001b). Damage can also be catalysed by environmental factors such as acidic conditions, heat and radiation (Lindahl 1993). Damage may take a variety of forms, including base changes and missing bases, lesions or breaks in one or both strands, denaturation, as well as inter- and intra-molecular cross-linking (Pääbo et al. 1989). As a result, most DNA recovered from archaeological contexts survives as fragments less than 500bp long (Cooper 1994; Pääbo et al. 1989). Cumulative DNA damage limits the potential for successful DNA research.

The advent of the polymerase chain reaction (PCR) by Kary Mullis in 1985 (Saiki et al. 1985) led to a proliferation of ancient DNA research. PCR enabled the amplification, or production of many copies, of ancient DNA fragments from degraded or very small samples of DNA. In simple terms, PCR works by mixing samples of extracted DNA, primers (synthesised DNA sequences used to initiate replication of desired fragments), thermostable polymerase and a solution of nucleotides in a three hour reaction of repetitive denaturing and re-annealing cycles to exponentially duplicate the DNA in a chain reaction (Pääbo et al. 1989). In theory, only one undamaged sequence need remain in a sample to be exponentially amplified into several million copies through PCR. However, the efficiency and accuracy, or fidelity, of the replicated PCR product sequences largely relies on the optimisation of the reaction conditions (Keohavong and Thilly 1989), and a variety of degradation processes and/or products can result in replication errors or inhibit amplification by PCR (Binladen et al. 2006; Cooper 1994; Pääbo et al. 1989). The successful application of PCR to a variety of museum specimens of extinct species such as the marsupial wolf (Thomas et al. 1989) and moa (Cooper et al. 2001; Cooper et al. 1992), has broadened the research potential of existing hard and soft tissue collections.

Publication of the successful extraction, cloning and sequencing of DNA from an ancient Egyptian mummy (Pääbo 1985) received widespread publicity and, it was hoped that the genetic relationship between Pharaonic families and dynasties could be elucidated. However, the results of this study were eventually called into question due to concerns about contamination (Cooper and Wayne 1998; Hagelberg and Clegg 1991). Since then, a number of other studies have attempted to extract and PCR amplify DNA from ancient Egyptian mummies with variable results, and debate has raged about the potential preservation of DNA in such material largely centred around the effects of the hot Egyptian climate on DNA (Gilbert et al. 2005b; Marota et al. 2002; Zink and Nerlich 2003; Zink and Nerlich 2005).

DNA can be used to address a diverse range of research questions, including phylogenetic relationships, population studies, migration, domestication, sex identification, kinship analysis and disease studies. Some results based on DNA research would not otherwise have been elucidated using traditional research approaches and methods. Although research prior to 1995 (Brown 2001) was somewhat unreliable due to a general lack of contamination controls and an evolving understanding of the limitations of the methods and materials used, today the samples required for DNA analysis are smaller, the methods better understood and the research questions more robust. With improved methods and an increase in facilities capable of undertaking DNA research, there is more demand on museums to provide samples for research, and conservators will inevitably become more involved not only in advising on the treatment history of collection materials and their subsequent viability for research purposes, but also in devising methods to

improve DNA preservation in newly acquired specimens.

Chemical treatments may affect DNA studies in a variety of ways. Although the threat of human DNA contamination by handling specimens is well known (Gilbert et al. 2005c; Malmström et al. 2005; Richards et al. 1993; Richards et al. 1995; Yang and Watt 2005), the risk of contamination with animal DNA from preparation and conservation treatments derived from animal sources has received relatively little attention (Cooper 1994; Nicholson et al. 2002). This demonstrates the lack of involvement of conservators in biochemical discourse and research to date. It has also been suggested that the presence of preparation and conservation treatment chemicals in specimens may inhibit PCR (Cooper 1994; Hall et al. 1992), so that DNA fails to be amplified, thereby indicating that DNA is extremely degraded in a specimen when in fact a chemical artefact has interfered with the reaction which otherwise may have been successful. It may also be possible that chemical treatment could induce cross-linking either between DNA and DNA or between DNA and protein, which could also affect the efficiency of PCR. Lastly and most importantly, chemical treatment may destroy DNA. For example, biochemists routinely use sodium hypochlorite bleach to eliminate surface-contaminating DNA on specimen samples (Kemp and Smith 2005), so it may be assumed that prolonged immersion in sodium hypochlorite bleach, sometimes used to whiten bone for exhibition, may adversely affect DNA preservation, rendering such treated specimens less suitable for DNA studies. Improved biochemical methods have been and may still be devised to circumvent some of these treatment-induced problems, such as the use of N-phenacylthiazolium bromide to release DNA bound up in sugar cross-links (Poinar et al. 1998).

Museums are increasingly solicited for tissue samples for DNA research projects. This growing interest in DNA studies and subsequent requests for samples has put increased pressure on collections and collection managers. As these materials are irreplaceable, and due to an increasing awareness of ethical issues surrounding human remains collections, their uses and appropriate handling, treatment and storage, much more caution is taken in providing samples. Furthermore, due to the fragmentary state of DNA in collection materials and the sensitive nature of the methods employed in DNA analysis, such as PCR, results cannot be guaranteed. A variety of methods have been suggested to screen for potential DNA recovery, such as histological characterisation (Colson et al. 1997; Götherström et al. 2002), collagen content (Götherström et al. 2002), amino acid racemization (Poinar 2002), and measuring the extent of modified DNA bases by gas chromatography/mass spectrometry (Höss et al. 1996; Poinar 2002). Although each of these methods has met with some success in determining proxies for DNA preservation, they also require destructive sampling to some degree, and their successful application cannot necessarily ensure success in DNA analyses. This leaves both biochemical researchers and museum professionals requiring further guidance for determining the viability

of collections material for sampling. In addition, the effects of chemical treatments on DNA are currently not well characterised, but it has recently been noted that museum prepared specimens displayed greater sequence variation than a number of archaeological specimens (Binladen et al. 2006), indicating that the materials and methods used in collections may have a negative effect on DNA preservation. A better understanding of the history of treatments and their effects could provide some assistance in identifying material best suited for DNA analysis.

DNA damage is a limiting factor to the success of ancient DNA studies, and the possible effects of preparation and conservation treatments on DNA in collection material needs to be taken into account in the future management of collections. To understand better the utility of existing collections and to inform future conservation treatment methodologies, research is needed to assess the effects of past preparation and conservation treatments on DNA as well as other biomolecules. As new biochemical methods and research questions are applied to collections, we need to ensure collection materials are maintained with the preservation of biomolecules in mind. This affects conservators perhaps more than any other profession involved in museum and collection management. Discussions about what constitutes best practice must be revisited to include biochemical preservation as our knowledge of the material develops. Regarding biological collections, it has been argued that "[t]he role of museum collections is to *conserve* scientifically important specimens so that they remain in *good condition* and *available* for *use*" (Pritchard and Kruse 1984: 253, emphasis in original), and that there is little justification for maintaining collections that are not useful for research (Williams 1999). Although preserving the research potential of material may not be viewed as the primary role of conservation in other disciplines, the principles of conservation require that the integrity of materials be preserved as best possible, and it would be negligent to disregard biomolecular preservation.

1.3. Project design and research questions

This project was designed to address the issues surrounding the effects of chemical preparation and conservation treatments on DNA preservation in museum collections. Any collection types that may contain human or animal remains were considered, and any preparation and conservation treatments potentially administered to both hard and soft tissues were taken into account. This research was undertaken from a museum conservation perspective, utilising biochemical methods with the intention of identifying treatments that are either damaging to DNA or promote DNA preservation in order to suggest appropriate measures to promote molecular preservation in conservation and collections care strategies. Investigating the potential effects past treatments have had on current collections may also help to inform conservators about the utility of their collections for biochemical research, so that they may be more effective in advising on sampling strategies.

The project consisted of four key components. Firstly, the existing literature related to the

effects of chemicals on DNA was reviewed to determine the utility of the methods already employed to investigate the subject. Secondly, a range of preparation and conservation treatments used on both human and animal soft and hard tissues were surveyed to identify chemicals used in the past and their methods of application to collection materials both in the field and in museums. Thirdly, a screening test was devised to assess the effects of many of the most commonly documented preparation and conservation treatments on short strands of DNA *in vitro* and to quantify the degree of strand breakage sustained by treatment. Lastly, the effects of ancient Egyptian mummification techniques, one of the earliest preparation and conservation treatments, were investigated briefly.

The three main research questions addressed by this study were:
1) What effect, if any, do preparation and conservation treatments have on DNA?
2) Based on these results, is it possible to predict the viability of DNA research using previously treated material?
3) Is it possible to suggest materials that are preferred for use based upon minimal effect, or possibly even promoting DNA preservation?

This research highlights the importance of developing conservation methods specifically aimed at the preservation of DNA in specimen collections, as several of the chemicals tested were found to cause DNA damage *in vitro*. It is hoped that the results of this study will be of use to biochemists and conservators, as well as curators, museum managers, archaeologists, biologists, students and other researchers, as better knowledge about the effects of conservation treatments on DNA may shed light on previously published data or sampled specimens and should affect future excavation and collection care strategies.

Chapter 2. Review of past studies regarding the effects of treatments on DNA

The first step in investigating the effects of preparation and conservation treatments on DNA was to review the existing literature on the subject. Several studies have addressed the effects of chemical treatments on the extraction and/or utility of DNA from collection materials for biochemical research. Most have focused on the effects of fixatives used for soft tissue preservation with the objective of extracting and analysing high molecular weight DNA. Two disciplines in particular have generated discrete bodies of literature on this subject, namely the medical pathology profession and biologists, particularly those involved in insect and arachnid studies. Both of these fields took a different approach to studying the problems associated with using prepared specimen material for DNA research. Medical pathology studies pursued methods to optimise DNA extraction and reliability, whereas biological studies focused on developing DNA preservation strategies.

2.1. Medical pathology studies

Much of the early research regarding the effects of fixatives on the utility of DNA for biochemical research originated from clinical and pathology studies, attempting to extract DNA from archived tissue samples that were typically formalin fixed and paraffin embedded. The results of many of these studies have largely been reviewed elsewhere in the biochemistry and medical pathology literature (Crisan and Mattson 1993; Srinivasan et al. 2002), but they are reviewed here with specific reference to their utility to conservation.

The medical pathology research has primarily been methodological and protocol driven rather than preservation driven. In the quest to determine whether archival fixed tissues could be utilised for biochemical analysis, methods to get round various preservation issues have been sought rather than identifying methods to preserve DNA within specimens. Research has been undertaken by biochemists with little involvement of preparators and conservators, so projects have been designed to assess the effects of fixatives on various biochemical *procedures*, such as PCR, with little regard to understanding the fundamental effects of fixatives on DNA. As a result, it has been determined that the success of DNA studies using fixed tissues relies upon selecting suitably sized sequences for study (Longy et al. 1997; Wong et al. 1998), using appropriate pre-digestion (Goelz et al. 1985; Jackson et al. 1990; Lehmann and Kreipe 2001; Smith et al. 1987) or pre-PCR restorative protocols (Bonin et al. 2003), undertaking additional purification measures to remove inhibitors such as small DNA fragments and histological stains or fixative residues (Kösel et al. 2001; Satoh et al. 1998), and using high quality *Taq* polymerase for PCR (Akalu and Reichardt 1999). All of these improvements were useful for dealing with the effects of fixatives but do not address the root cause of DNA damage resulting

from fixation. Although developing methods to cope with the preservational vagaries of existing collections is important, it does not necessarily inform or improve the lot of expanding collections.

Many of the medical pathology studies have focused on the effects of DNA specifically on PCR. Although of importance to biochemists, the design of several studies does not account for actual damage to DNA resulting from fixation. Many studies only report amplification results, and do not assess the quality or quantity of DNA extracted from samples prior to PCR (Barnes et al. 2000; Ben-Ezra et al. 1991; Bonin et al. 2003; Bonin et al. 2005; Satoh et al. 1998). This is of little use in assessing the effects of fixation on DNA because PCR was designed as a tool for use on samples with poor DNA survival to generate many copies of a target sequence, thus damage caused by chemical treatment will be compensated for by PCR. Successful amplification by PCR does not mean the DNA in a sample is necessarily well preserved or that treatments administered to it in the past were not damaging. Furthermore, damage is not limited to DNA loss or breakage, but base changes resulting from chemical treatment could also occur, which can only be investigated by sequencing analysis (Longy et al. 1997; Wong et al. 1998). Additionally, failure to amplify target sequences may be due to PCR inhibition, possibly resulting from chemical residues, which if not identified would be misinterpreted as extreme DNA damage.

A handful of medical pathology PCR based studies have reported the quality of DNA extracted from samples prior to amplification (Jackson et al. 1990; Kilpatrick 2002; Kösel et al. 2001; Matsuo et al. 1999). Of the papers reviewed, only two were designed in a manner that all variables that could potentially cause DNA damage were accounted for, and these papers addressed the effects of buffer solutions (Kilpatrick 2002) and controlled environmental conditions (Matsuo et al. 1999), but not fixatives. One study that used samples prepared specifically for experimental purposes to test the effects of fixatives (Kösel et al. 2001) provided a full account of methods and materials used for fixation, but did not report on the conditions surrounding tissue procurement or paraffin embedding. Another study (Jackson et al. 1990) utilised some freshly collected tissues, but also used several archival tissues, for which no details regarding their preparation were given. This lack of reporting sample preparation procedures is a recurring problem in the medical pathology literature, as it precludes the identification of materials and methods responsible for DNA damage. Identifying archival tissue samples with a reliable treatment and storage history is fundamental to assessing the effects of treatments on DNA over time.

As a by-product of research undertaken to optimise methods for DNA analysis, it was noted that it may be inferred that the preservation of DNA in fixed tissues depends upon many of the same

factors that determine the preservation of fixed tissue morphology, such as whether a tissue was collected as a surgical biopsy or postmortem (Bonin et al. 2003), the amount of time passed between tissue collection and fixation or cold storage (Goelz et al. 1985), the fixative used (Ben-Ezra et al. 1991; Goelz et al. 1985; Kösel et al. 2001), the temperature of fixation (Tokuda et al. 1990), the size of specimen and volume of fixative (Kilpatrick 2002), the thoroughness of fixation (Barnes et al. 2000), maintenance of storage solutions (Barnes et al. 2000), the duration of fixation (Tokuda et al. 1990), and the conditions and duration of storage (Hajduk 1999; Matsuo et al. 1999; Matsuo et al. 1995). The effects of embalming fluids on postmortem samples should also be considered. In spite of gaining a basic understanding of the issues surrounding DNA preservation through these studies, little is understood about the effects of each of these independent variables, each of which is worthy of systematic study.

In summary, due to a number of methodological problems in the medical pathology literature regarding the potential effects of fixatives on DNA, the results of much of this research are not useful from a conservation point of view to assess either the utility of existing collections based on their treatment history or to devise future collections care strategies to optimise DNA preservation. Incomplete preparation details were often provided for tissue samples prepared specifically for the purposes of DNA research, and many of the archived tissues had been fixed and prepared using unknown protocols and stored in undisclosed conditions for indefinite periods of time. In addition to this lack of information regarding sample preparation, in an effort to improve results, most studies made adjustments to previously published extraction protocols, ultimately making comparisons about the effects of treatments on DNA between studies difficult, as it cannot reliably be determined if differences in results were due to the samples or to the protocols used. Furthermore, few studies were performed in such a way as to determine the effects of chemical treatments on DNA, but focused instead on improving DNA extraction and amplification techniques, accepting the state of preservation in past collections, but with little thought given to improving matters for future collection specimens.

2.2. Biological studies

The biological sciences have been the source of much of the systematic research regarding the effects of preparation and conservation treatments on DNA in collection materials (see Table 1 for a summary of published works). With an appreciation for the challenges associated with fieldwork and the collection of live specimens (Gurdebeke and Maelfait 2002; Reiss et al. 1995) as well as knowledge that even some recently collected specimens are already unsuitable for DNA analysis (Quicke et al. 1999), biological researchers were quick to realise that methods and materials used on newly collected specimens must facilitate DNA preservation. The experimental design of many of these biology based studies is considerably more robust than that of the preceding medical pathology studies, acknowledging that earlier research was selective in the methods tested and the results were often inconsistent with each other (Post et

al. 1993).

Improvements on research design in biological studies compared to the medical pathology research include the testing of a variety of chemicals and storage temperatures under controlled environmental conditions over different lengths of time, enabling the true comparison of different chemicals and conditions over time. Another improvement was the realisation that both the quality and quantity of extracted DNA must be reported as well as the results of any other biochemical methods such as PCR amplification. Reporting only the yield of DNA from a sample is inadequate, as even a high yield of DNA may be too fragmented in nature to be of use for some biochemical methods. Additionally, knowing that a high yield of high quality extracted DNA failed to yield PCR products under optimised amplification conditions can enable the identification of PCR inhibitors, for example. Furthermore, only reporting whether extracted DNA was successful for use in PCR does not equate to a treatment causing no damage to DNA, as well illustrated by data provided by Carter (2003), specifically that cryopreserved material stored for 20 months yielded approximately 79±41 μg/ml extracted DNA and 80% IMS stored for over 15 years yielded approximately 9±8 μg/ml extracted DNA, and both were used to successfully amplify a 500bp 16S mitochondrial DNA fragment. If cryopreservation is assumed as a baseline for optimal preservation (effectively "no damage"), this implies that a decrease in yield by almost 90% of considerably fragmented DNA can still result in successful amplification by PCR.

Reference	Sample material	Killing method	Sample preparation	Insect: fluid ratio	Pre-extraction time stored	Pre-extraction temp	Sample size	DNA yield (rounded)	Post-extr. time stored	Post-extr. temp	Methods, results and problems
Post et al. 1993	Flies	Direct	Cryopreservation	NA	142-372 days	-20°C then liquid nitrogen	Individual flies for yield, 5 flies combined for condition; number of replicates varied from 2-15	974-1326ng	NA	-40°C	**Extraction protocol:** Modification of Coen et al 1982. Frozen flies not pre-treated. Flies preserved in liquid rehydrated in TE for 10 min at RT. Dried flies rehydrated in 2XSSC and 1% SDS for 20 min, rinsed in TE for 5 min, all at RT. Individual flies homogenized in 100µl Bender buffer (0.1M NaCl, 0.2M sucrose, 0.1 M Tris-HCl, 0.05M EDTA pH 9.1 and 0.5% SDS in water) with 1% DEPC, incubated at 65°C for 1 hour. 15µl 8M K-acetate added at 4°C and incubated on ice for 45 min. Precipitate spun down for 5 min and supernatant added to 2X its volume of 100% ethanol and incubated at RT for 5 min. Spun down for 10 min and supernatant discarded. DNA precipitate redissolved in 20µl of 0.1X SSC and RNase (10µg/ml) overnight at 4°C. **Analysis methods:** Total DNA yield measured by fluorescence of 4µl DNA extract with Hoechst 33258 using a Hoeffer TKO 100 dedicated minifluorometer. Quality of undigested DNA determined by 15µl sample undigested on minigel at 70V in 0.8% agarose gel, photographed with Polaroid type 57 film using a Polaroid MP4 Land Camera fitted with a Wratten 22A filter. Photos scanned using a Joyce-Lobel Chromoscan 3 densitometer, background adjusted and 3 size ranges calculated. %DNA shearing calculated using densitometry data. **Results:** Highest DNA yields from flies stored in liquid nitrogen, 100% ethanol at 4°C and dried over silica gel; lowest yield was pinned (these were also the oldest specimens), formal saline and Carnoy's solution. Least degraded DNA was from frozen samples, silica gel also good, ethanol OK; Carnoy's bad. Although propanol gave a high yield, DNA very degraded. **Problems:** Unknown if pinned specimens killed first and/or how. Samples treated for variable lengths of time (different RT?), so comparison not ideal.
		Direct	Carnoy's solution (ethanol:acetic acid, 3:1)		142 days	4°C		701-974ng			
		Direct	Carnoy's solution (ethanol:acetic acid, 3:1)		142 days	RT 2 weeks then 4°C		652-967ng			
		Direct	Carnoy's solution (ethanol:acetic acid, 3:1)		122 days	RT		367-424ng			
		Direct	10% formal saline		368 days	4°C		0ng			
		Direct	10% formal saline		368 days	RT 2 weeks then 4°C		0ng			
		Direct	10% formal saline		122 days	RT		0-42ng			
		Direct	Silica gel desiccation in the sun		2017 days	RT		860-1099ng			
		Direct	Silica gel desiccation in the sun		137 days	4°C		1153-1333ng			
		Direct	Methanol (60%)		141 days	4°C		820-1036ng			
		Direct	Methanol (80%)		141 days	4°C		416-556ng			
		Direct	Methanol (100%)		141 days	4°C		519-586ng			
		Direct	Ethanol (60%)		128 days	4°C		1199-1324ng			
		Direct	Ethanol (80%)		136 days	-20°C		485-687ng			
		Direct	Ethanol (80%)		129-372 days	4°C		675-1150ng			
		Direct	Ethanol (80%)		121 days	RT		618-925ng			
		Direct	Ethanol (100%)		136 days	4°C		1403-1659ng			
		Direct	Propan-2-ol (60%)		122 days	4°C		470-629ng			
		Direct	Propan-2-ol (80%)		120 days	-20°C		805-917ng			
		Direct	Propan-2-ol (80%)		122 days	4°C		980-1070ng			
		Direct	Propan-2-ol (80%)		137 days	RT 2 weeks then 4°C		484-702ng			
		Direct	Propan-2-ol (80%)		121 days	RT		542-585ng			
		Direct	Propan-2-ol (100%)		127 days	4°C		718-1034ng			
		Unknown	Pinned		2919-4545 days	RT?		0ng			

Reference	Sample material	Killing method	Sample preparation	Insect: fluid ratio	Pre-extraction time stored	Pre-extraction temp	Sample size	DNA yield (rounded)	Post-extr. time stored	Post-extr. temp	Methods, results and problems
Reiss et al. 1995	Beetles	Unknown	Ethyl acetate (tissue paper impregnated with)	NA	11-144 days	RT	20 per treatment stored together		NA	NA	**Extraction protocol:** Modification of Vogler et al 1993. Homogenized in 450 µl DNA isolation buffer containing 1 µg/ml RNase A, and 0.3 mg/ml Proteinase K, incubated 1 hour at 60°C and centrifuged at 300xg for 1 min. Supernatant extracted with phenol, with 1:1 phenol:chloroform, and with chloroform. Precipitated aqueous phase by adding 1/4 volume 10M ammonium acetate and 2 volumes ethanol. Stored for at least 1 hour at -20°C, centrifuged 30 min at 16,000xg. Pellet washed in 70% ethanol, air dried and resuspended in 100µl TE (10mM Tris, 0.1 mM EDTA). **Analysis methods:** Quality of undigested DNA determined on 0.8% agarose gel and quantity determined by densitometry. Photographs scanned by Hewlett Packard Scanner, image analyzed using Collage. Digested with restriction enzymes, and 742bp PCR product generated of mt cytochrome oxidase III gene. **Results:** For long term storage, cryopreservation best; short term ethanol or homogenized specimens in buffer. HMW DNA extracted from ethanol, buffer and cryopreserved samples, but not ethyl acetate or Carnoy's. Specimens left whole in buffer degraded, but homogenized specimens yielded HMW DNA. Ethanol samples degraded after 73 days, Carnoy's by 18 days, buffer showed irregular damage possibly due to poor homogenization, and cryopreserved material not degraded significantly. PCR products obtained from ethanol, liquid nitrogen and homogenized buffer; PCR failed for Carnoy, ethyl acetate and whole specimens in buffer. **Problems:** Unknown: killing method, if insects processed as individuals or batches, and yield not known for each treatment (average of 1-3ng/µg tissue reported).
		Unknown	Ethanol (95%)		11-144 days	RT					
		Unknown	Carnoy's solution (methanol:acetic acid, 3:1)		11-144 days	RT					
		Unknown	DNA isolation buffer (100mM Tris, pH 8.0, 50 mM EDTA, 160 mM sucrose pH 8.0, and 1.0% SDS) half insects whole, half homogenised		11-144 days	RT					
		Unknown	Cryopreservation		11-144 days	-80°C (liquid nitrogen)					
A'Hara et al. 1998	Spiders	Direct?	Ethylene glycol	NA	3 weeks	RT	Individual spider carapace (frozen in nitrogen to enable dissection)		over 1 month	4C and -20°C	**Extraction protocol:** As per Cheung et al 1993. Carapace homogenized and added 500µl chilled DNA extraction buffer (200mM Tris-HCl (pH 8.0), 70mM EDTA, 2M NaCl, 20mM sodium metabisulphite) and 90µl 5% sarcosyl solution, ground and incubated for 1 hour at 65°C with occasional mixing. Centrifuged at 16,000xg for 3 min. Supernatant precipitated with 90µl 10M ammonium acetate and 500µl chilled isopropanol, mixed and stored at -20°C for 2 hours. Centrifuged at 16,000xg for 10 min, supernatant removed and pellet washed with 400µl 70% ethanol. Air dried and resuspended in 50µl sterile water heated to 60°C for 1 hour. **Analysis methods:** Extracted DNA examined on 1% agarose gel, and PCR RAPD reactions carried out with stored DNA over 1 month, visualised on 1.5% agarose gel. **Results:** Extracted DNA of poor quality from ethylene glycol and 70% ethanol samples; storage at -80°C was best for preserving specimens up to 1 year prior to extraction. Storage of extracted DNA best at -20°C, variable results for 4°C. **Problems:** Unknown: killing method, and if PCR for cryopreserved material only.
		Direct?	Ethanol (70%)		3 weeks	RT					
		Direct?	Cryopreservation		3 weeks	-80°C (liquid nitrogen)					
Fukatsu 1999	Pea aphid and its symbiotic bacterium	Direct?	Acetone (50, 70, 80, 90 and 100%)	Less than 1/20 (must be	6 months	RT	NA (several insects implied)		NA	NA	**Extraction protocol:** Individual insects homogenized in 500µl lysis buffer (50mM Tris-HCl (pH 8.0), 10mM EDTA, 0.5% SDS, 0.8 mg/ml Proteinase K) at RT, and incubated overnight at 55°C. Lysate extracted twice with phenol and once with chloroform. Precipitated with isopropanol, spun down, rinsed with 80% ethanol, air dried and dissolved with 20µl TE (10mM Tris-HCl (pH 8.0), 0.1mM EDTA).
			Ethanol (50, 70, 80, 90 and 100%)		6 months	RT					
			Methanol		6 months	RT					

Reference	Sample material	Killing method	Sample preparation	Insect: fluid ratio	Pre-extraction time stored	Pre-extraction temp	Sample size	DNA yield (rounded)	Post-extr. time stored	Post-extr. temp	Methods, results and problems
			2-propanol	greater than 1/3)	6 months	RT					**Analysis methods:** Extracted DNA examined on 1.2% agarose gel, and purity confirmed by measuring absorption spectra. PCR of 1.6kb mtrDNA fragment of host and 1.5kb fragment of 16S rDNA, SDS-PAGE and immunoblotting, histology, immunohistochemistry, in situ hybridization. **Results:** Acetone best preservative overall. After 6 months, acetone, ethanol, 2-propanol, diethyl ether and ethyl acetate all good. Methanol failed. Chloroform also poor possibly due to poor penetration - insects floated. PCR results were similar for both host and endosymbiont except for chloroform, where symbiont did not amplify. Further investigation into ethanol and acetone indicated that 90%+ ethanol OK, and 50%+ acetone OK for 6 months. Acetone preserved specimens, had good extraction and host amplification up to 2 years. Other insects tested after storage in acetone for up to 5 years and 4 months, with good extraction and PCR of host for all (symbiont weak or failed at 5 years 3 months). Suggested changing hydrated solvents. **Problems:** Unknown: killing method.
			Diethyl ether		6 months	RT					
			Ethyl acetate		6 months	RT					
			Chloroform		6 months	RT					
Carvalho and Vieira 2000	Ants	95% ethanol 48 hours?	Cryopreservation	NA	90, 210 and 360 days	-70°C	Assumed to be individual ant heads (4 replicates)	3.9, 4.2, 3.6µg	NA	-20°C	**Extraction protocol:** Modification of Cheung et al. 1993. Head placed in desiccator under vacuum for 30 min and homogenised in 200µl extraction buffer (200mM Tris-HCl, pH 8.0, 2M NaCl and 70mM EDTA). Added 50µl 5% sarcosyl, homogenized again and incubated at 65°C for 30 min. Centrifuged at 10,000g for 15 min. Supernatant precipitated by adding 110µl 10M ammonium acetate and 250µl cold isopropanol, stored overnight at 4°C. Centrifuged at 10,000g for 15 min, and pellet washed twice with 70% ethanol, air dried and resuspended in 50µl TE (10mM Tris and 1mM EDTA, pH 8.0) containing 10µg/ml RNase. **Analysis methods:** Extracted DNA quantified by fluorescence with Hoechst 33258 and DyNA Quant 200 minifluorimeter. Quality assessed on 0.8% agarose gel, photographed (Polaroid MP4 camera and Polaroid 667 film), scanned with densitometer (Bio-Rad model 620). RAPD of >9.4kb fragment on 1.5% agarose gel. **Results:** Best method was -70, followed by 95% ethanol at -20°C, 95% ethanol at 4°C, silica gel, buffer; 95% ethanol at RT not recommended. Yield: Best preservation provided by silica gel at RT. 95% ethanol at RT had lower yields of DNA at all times. Preservation better in 95% ethanol at 4°C and -20°C. All methods OK up to 7 months, and all but 95% ethanol at RT OK for up to 12 months. Quality: All methods resulted in increased damage after 210 days, with silica gel and buffer samples very degraded, and 95% ethanol at RT most degraded after 360 days. RAPD analysis indicated that 95% ethanol at RT not suitable (missing bands). Statistical analysis: conditions over time affect quality (not quantity). **Problems:** Unknown: killing method, sample size or if DNA yield is per head.
		95% ethanol 48 hours?	95% ethanol		90, 210 and 360 days	-20°C		3.9, 5.0, 3.6 µg			
		95% ethanol 48 hours?	95% ethanol		90, 210 and 360 days	RT		2.0, 2.1, 0.4 µg			
		95% ethanol 48 hours?	95% ethanol		90, 210 and 360 days	4°C		3.1, 3.4, 4.1µg			
		95% ethanol 48 hours?	Silica gel		90, 210 and 360 days	RT		4.8, 4.4, 4.8µg			
		95% ethanol 48 hours?	Buffer (0.25M EDTA, 2.5% SDS, 0.5M Tris-HCl, pH 9.2)		90, 210 and 360 days	RT		4.3, 3.7, 3.9µg			
Dean and Ballard	Flies	Cyanide (7-9 min)	Pinned (with and without naphthalene)	NA	2 years	RT (25°C)	5 individuals		NA	NA	**Extraction protocol:** Individual insects homogenized and cells lysed before Proteinase K and RNase A digestion. Protein precipitated with cold (4C)

Reference	Sample material	Killing method	Sample preparation	Insect: fluid ratio	Pre-extraction time stored	Pre-extraction temp	Sample size	DNA yield (rounded)	Post-extr. time stored	Post-extr. temp	Methods, results and problems
2001		Ethyl acetate (7-9 min)	Pinned (with and without naphthalene)		2 years	RT (25°C)	for extraction, 3 for PCR				isopropanol. Puregene DNA isolation kit D-7000A, using 1/3 standard volumes. DNA precipitates washed, dried and rehydrated with 20µl autoclaved ddH2O. **Analysis methods:** Extraction yields run on 1.5% agarose gel, quantified using GeneQuant spectrophotometer. Amplification of 291, 959, 1332 and 1822bp fragments by PCR and visualised on 1.5% agarose gel. **Results:** Best killing method = cyanide, worst = 70% ethanol. Naphthalene did not reduce DNA quality in terms of yield or PCR after 2 years. After 2 years, all killing methods showed some degradation and PCR success decreased. Sequencing was 96% accurate from PCRs with variable success. Statistical analysis = killing method significantly affected DNA yield, and duration of storage significantly affected PCR. **Problems:** Statistical analysis questionable re: effects of "time".
		Freezing (-20°C, 7-9 min)	Pinned (with and without naphthalene)		2 years	RT (25°C)					
		Ethanol (70% at RT, 7-9 min)	Pinned (with and without naphthalene)		2 years	RT (25°C)					
Gurdebeke and Maelfait 2002	Spiders	NA	Ethanol (70%)	NA	2 months	RT	1 pair of legs (8 replicates)	60±34ng/µl	NA	NA	**Extraction protocol:** PureGene DNA isolation kit (type D-5000A, Gentra Systems) **Analysis methods:** Extracted DNA quantified with spectrophotometer, and RAPD. **Results:** Of the solutions tested, ethanol best and acetic acid:TE worst. Best yield: Cryopreservation, ethanol and formaldehyde OK, no DNA extracted from acetic acid-TE. Best quality: Cryopreservation and ethanol OK - formaldehyde failed. When used in live traps, 96% ethanol in funnel traps recommended for catching specimens and preserving their DNA (live traps with -20°C best but few specimens caught); using 85% and 75% ethanol resulted in degraded DNA. **Problems:** Although killing method not reported, equalised across samples as legs from same specimen used in each solution.
		NA	1:1 acetic acid:TE		2 months	RT		0ng/µl			
		NA	4% formaldehyde		2 months	RT		53±106ng/µl			
		NA	Cryopreservation		2 months	-20°C		129±53ng/µl			
Carter 2003	Wood-louse		Cryopreservation	NA	20 months	-30°C	NA	79±41µg/ml	NA	NA	**Extraction protocol:** Modified CTAB method used. Tissue placed in 200µl TE (0.05M EDTA, 0.1M Tris-HCl pH 8.0) and homogenised, then added 200µl TE, 50µl 20% (w/v) SDS and 15µl Proteinase K (2mg/ml) and incubated for 2 hours at 60°C. Added 200µl 5M NaCl and 1/10 volume 10% (w/v) CTAB, mixed and incubated for 15 minutes at 60°C. 20µl RNaseA (10mg/ml) added, incubated at 37°C for 30 min. Equal volume of cold chloroform:isoamyl alcohol (24:1) added, mixed, incubated at RT for 5 min, and upper aqueous layer removed. Promega Protein Precipitation solution (1/3 volume) added to aqueous phase, vortexed and centrifuged for 5 min. Precipitated with 1 volume cold isopropanol, frozen overnight, centrifuged 10 min. Isopropanol removed, air dried 10 min and DNA resuspended in 200µl TE. **Analysis methods:** Extracted DNA run on 1% agarose gel. Yield calculated by image analysis with Scion Image software. Restriction endonuclease digestion. Nuclease S1 treatment. PCR run on 1% agarose gel. **Results:** Cryopreservation best. Ethanol preservation overall good (4°C better than RT), with some degradation by 24 months. Changing fluid and addition of EDTA may result in fragmentation. 100% IMS OK. 80% IMS, ethyl acetate, formaldehyde and Steedman's fluids poor. When PCR successful, both nuclear and mt DNA amplified except for 15+yr 80% IMS sample (only mt). Rehydration is damaging. **Problems:** Unknown: full details of conditions and solutions used (no formula for
			Ethanol (100%)		1 day, month or week?	RT?		54±15µg/ml			
		Direct?	Ethanol		3 months	RT		49±6µg/ml			
		Direct?	Ethanol		24 months	RT		55±8µg/ml			
		Direct?	Ethanol		12 months	4°C		67±19µg/ml			
		Direct?	Ethanol (fluid changed after 1 week)		13 months and 1 week	RT?		53±22µg/ml			
			Ethanol + trace EDTA (100µM)		15 months	RT?		28±9µg/ml			
			IMS (100%)		15-18 months	RT?		53±21µg/ml			
			Ethanol		~15-18 months	RT?		56±8µg/ml			
			IMS (80%)		2 months	RT?		40±17µg/ml			
			IMS (80%)		~15-18 months	RT?		25±14µg/ml			
			IMS (80%)		15+ years	RT?		9±8µg/ml			
			Propylene glycol		12 months	RT?		23±7µg/ml			

Reference	Sample material	Killing method	Sample preparation	Insect: fluid ratio	Pre-extraction time stored	Pre-extraction temp	Sample size	DNA yield (rounded)	Post-extr. time stored	Post-extr. temp	Methods, results and problems
			2-ethoxy ethanol		12 months	RT?		20±9µg/ml			Steedman's solution). Assumed ethanol used is 100% in all cases.
			Ethyl acetate		12 months	RT?		0µg/ml			
			Formaldehyde (4%)		12 months	RT?		<5µg/ml			
			Steedman's solution		12 months	RT?		<5µg/ml			
			Ethanol (dried)		18 months	RT?		62±12µg/ml			
			Ethanol (rehydrated 4% Decon90 24 hours)		18 months	RT?		31±5µg/ml			
			IMS (dried)		18 months	RT?		38±9µg/ml			
			IMS (rehydrated 4% Decon90 24 hours)		18 months	RT?		32±16µg/ml			
			IMS (80%, dried)		18 months	RT?		25±14µg/ml			
			IMS (80%, rehydrated 4% Decon90 24 hours)		18 months	RT?		24±26µg/ml			
Tayutivut-ikul et al 2003	Silkworm moths	NA	Cryopreservation (-20°C)	NA	6 months	-20°C	Thoraces of individual moths		NA	NA	**Extraction protocol:** Modification of Tuda et al. 1995. Individual thoraces were either ground in liquid nitrogen and transferred to lysis buffer or ground directly in lysis buffer. Following grinding, individual samples were homogenized in 500µl lysis buffer (50mM Tris-HCl, pH 8.0, 10mM EDTA, 0.5% SDS, 400µg/ml Proteinase K, 100mg/ml RNase A), and incubated at 55°C for 3 hours. Lysate extracted twice with phenol-chloroform-isoamyl alcohol (25:24:1) and once with chloroform-isoamyl alcohol (24:1). Added 40µl 3M sodium acetate, DNA precipitated by absolute ethanol, centrifuged, twice washed with chilled 70% ethanol and dissolved in 50µl TE buffer (10mM Tris-HCl, pH 8.0, 0.1mM EDTA). **Analysis methods:** Extracted DNA on 1% agarose gel. Amplified 1517bp fragment and PCR products on 1% agarose gel. RAPD on 4% agarose gel. **Results:** Recommends short term use of 70% ethanol. Extraction not affected by grinding under liquid nitrogen v. grinding directly in lysis buffer. PCR for -80°C and -20°C most like fresh specimens and 70% ethanol and hot-air dried specimens yielded lower amounts of DNA. RAPD result best for -80°C. **Problems:** Killing method, storage temp unknown. Extracted DNA not quantified.
		NA	Cryopreservation (-80°C)		6 months	-80°C					
		NA	Ethanol (70%)		6 months	RT?					
		NA	Rapid hot air drying (60°C)		6 months	RT?					
Rey et al 2004	Water mites	NA	Cryopreservation	NA	1 week and 4 months	-20°C	Single specimens		NA	NA	**Extraction protocol:** Modification of CTAB by Doyle and Doyle 1987. Specimens washed in distilled water, crushed with pipette tip in 700µl CTAB buffer (2% CTAB, 1.4M NaCl., 0.2M EDTA, 0.1M Tris-HCl and 0.4% β-mercaptoethanol, pH 8.0) and 100µg/ml Proteinase K, incubated overnight at 56°C. DNA extracted using phenol-chloroform-isoamyl alcohol followed by isopropanol precipitation. Resuspended in TE (10mM Tris-HCl, 0.1mM EDTA, pH 7.5), and purified using a modified silica method (Boom et al. 1990). **Analysis methods:** Percentage of successful extractions provided. PCR of >700bp
		NA	Koenike's fluid (45% water, 45% glycerine, 10% glacial acetic acid)		4 months, 10 months, and 25 years	RT?					
		NA	Ethanol (70%)		4 months, 10 months and 15 years	RT?					

Reference	Sample material	Killing method	Sample preparation	Insect: fluid ratio	Pre-extraction time stored	Pre-extraction temp	Sample size	DNA yield (rounded)	Post-extr. time stored	Post-extr. temp	Methods, results and problems
		NA	Angelier's fluid (1% anhydrous chromic acid, 98% water, 1% glacial acid acetic)		4 months, 10 months, and 25 years	RT?					fragments, on 2% agarose gel. Sequencing. **Results:** Angelier's recommended for short term use (3 months) although ethanol performed well. -20°C was best for up to 4 months (then no result). Alcohol, Koenike's and Angelier's preservation was variable (25 year old specimens initially stored in Angelier's fluid and transferred to Koenike's). **Problems:** Extraction results not given. No sequencing results provided. Uncertain if treatment history of older specimens is reliable.
Vink et al 2005	Spiders and scorpions (spider data summa-rised)	Freezing	Ethanol (95%)	~1:8	6 weeks	40°C	Whole spider and scorpion (3 replicates). DNA extracted from left legs 3 and 4 of spiders and left leg 4 patella of each scorpion.		NA	-20°C	**Extraction protocol:** Used DNeasy kit. Dried down using a Savant Speed Vac and resuspended in 100µl AE elution buffer. **Analysis methods:** Yield and quality compared by electrophoresis of extractions on 0.8% agarose minigels, photographed on an Eagle Eye II system. Quality also assessed by PCR of mtDNA cytochrome oxidase subunit 1058bp (COI) and nuDNA 800-3000bp actin fragments. Sequencing. **Results:** PCR worked for all treatments, and sequencing OK. RNAlater and propylene glycol were significantly better than all other preservatives tested (no significant difference between the two). No significant difference between 95% and 70% ethanol, and initial saturation in 95% followed by storage in 70% ethanol was not significantly worse than other ethanol treatments. Least effective was 70% transferred to 95% ethanol. Two additional suboptimal treatments of multiple specimens together in either 95% ethanol or 70% with transfer to 95% ethanol were similar to their regular counterparts. Best temperatures for storage found to be -20 or -80°C - recommends storage at -20 ASAP. Degradation apparent after 5 days. **Problems:** Extraction not quantified. Sequencing data not provided.
		Freezing	Ethanol (95%)		6 weeks	RT (19-24°C)					
		Freezing	Ethanol (95%)		6 weeks	2-4°C					
		Freezing	Ethanol (95%)		6 weeks	-20°C					
		Freezing	Ethanol (95%)		6 weeks	-80°C					
		Freezing	Ethanol (70%)		6 weeks	40°C					
		Freezing	Ethanol (70%)		6 weeks	RT (19-24°C)					
		Freezing	Ethanol (70%)		6 weeks	2-4°C					
		Freezing	Ethanol (70%)		6 weeks	-20°C					
		Freezing	Ethanol (70%)		6 weeks	-80°C					
		Freezing	Ethanol (95% 1 day, 70% 6 weeks)		6 weeks	40°C					
		Freezing	Ethanol (95% 1 day, 70% 6 weeks)		6 weeks	RT (19-24°C)					
		Freezing	Ethanol (95% 1 day, 70% 6 weeks)		6 weeks	2-4°C					
		Freezing	Ethanol (95% 1 day, 70% 6 weeks)		6 weeks	-20°C					
		Freezing	Ethanol (95% 1 day, 70% 6 weeks)		6 weeks	-80°C					
		Freezing	Ethanol (70% 1 week, 95% 5 weeks)		6 weeks	40°C					
		Freezing	Ethanol (70% 1 week, 95% 5 weeks)		6 weeks	RT (19-24°C)					
		Freezing	Ethanol (70% 1 week, 95% 5 weeks)		6 weeks	2-4°C					
		Freezing	Ethanol (70% 1 week, 95% 5 weeks)		6 weeks	-20°C					

Reference	Sample material	Killing method	Sample preparation	Insect: fluid ratio	Pre-extraction time stored	Pre-extraction temp	Sample size	DNA yield (rounded)	Post-extr. time stored	Post-extr. temp	Methods, results and problems
		Freezing	Ethanol (70% 1 week, 95% 5 weeks)		6 weeks	-80°C					
		Freezing	99.5%+ propylene glycol (6 weeks, 95% ethanol 1 day at 4°C)		6 weeks	40°C					
		Freezing	99.5%+ propylene glycol (6 weeks, 95% ethanol 1 day at 4°C)		6 weeks	RT (19-24°C)					
		Freezing	99.5%+ propylene glycol (6 weeks, 95% ethanol 1 day at 4°C)		6 weeks	2-4°C					
		Freezing	99.5%+ propylene glycol (6 weeks, 95% ethanol 1 day at 4°C)		6 weeks	-20°C					
		Freezing	99.5%+ propylene glycol (6 weeks, 95% ethanol 1 day at 4°C)		6 weeks	-80°C					
		Freezing	RNAlater		6 weeks	40°C					
		Freezing	RNAlater		6 weeks	RT (19-24°C)					
		Freezing	RNAlater		6 weeks	2-4°C					
		Freezing	RNAlater		6 weeks	-20°C					
		Freezing	RNAlater		6 weeks	-80°C					
Mtambo et al 2006	Ticks	Direct	Dried by refrigeration	NA	5 years	4°C/RT	Individuals and individual legs		NA	-20°C	**Extraction protocol:** 70% ethanol specimens washed in tap water, rinsed in TE (10mM Tris-HCl (pH 8.0), 1mM EDTA). Dried specimens washed in alcohol, tap water and rehydrated in TE for 6 hours. Specimens cut into 6-8 pieces and each piece put into 1.5ml tube. 3 extraction methods used: 1) TE buffer - 30µl TE added. 2) Collins et al 1987 - add 50µl heat activated buffer (0.08M NaCl; 0.06M EDTA (pH 8.0, NaOH adjusted); 0.10M Tris-HCl (pH 8.6); 0.5% SDS; 0.16M sucrose). Incubate at 65°C for 30 min, then add 7µl 8M KAc. Ice 30 min, centrifuge at 12,000g for 10 min at 4°C. To supernatant add 100µl cold absolute ethanol, ice for 5 min, centrifuge 16,000g for 20 min. Wash pellet with 150µl cold (-20°C) 70% ethanol, absolute ethanol, remove supernatant and air dry. Resuspend in 25µl TE. 3) Boom et al 1990, 1999 - add 250µl buffer (1M Tris-HCl, 0.5M EDTA, 6M guanidinium hydrochloride, 0.5% w/v Triton X-100) and 250µl milliQ water. Add 50µl Proteinase K and incubate overnight at 60°C with shaking. Add 40µl diatomaceous earth suspension, incubate 37°C for 1 hour with shaking. Remove supernatant, wash pellet 2X with 900µl 70% chilled ethanol, wash with 900µl acetone, dry at 50°C for 20 min. Add 90µl TE, incubate at 60°C for 20 min with
		Direct	Ethanol (70%)		10 years	RT					
		Direct	Cryopreservation		2 years	-80°C (transit at -150°C)					
		70% ethanol	Refrigerated with silica gel		1 year	4°C					

Reference	Sample material	Killing method	Sample preparation	Insect: fluid ratio	Pre-extraction time stored	Pre-extraction temp	Sample size	DNA yield (rounded)	Post-extr. time stored	Post-extr. temp	Methods, results and problems
											shaking. Spin down. **Analysis methods:** PCR **Results:** Refrigerated specimens best preserved, followed by 70% ethanol and cryopreservation. Dried specimens were poorly preserved. Extraction method 1 failed for all treatments, ad method 3 had a significantly higher success rate over method 2. 2 genes had different PCR success rates. **Problems:** Samples stored for different lengths of time.
Mandrioli et al 2006	Cabbage moths	Direct?	Dried by silica gel (16 hours)	NA	2 months, 2 years	RT	Whole individuals (5 replicates)		NA	NA	**Extraction protocol:** Individuals homogenized in S-EDTA buffer (0.1M NaCl, 50mM EDTA, pH 8.0), added 1% SDS and 100µg/ml Proteinase K, incubated at 55°C overnight. 1 volume phenol-chloroform-isoamylic alcohol (25:24:1) added, stirred and centrifuged at 12,000g for 5 min. Repeated. Digested with RNase (100µg/ml) for 30 min at 37°C, ethanol precipitated, washed 3 times with 70% ethanol, air dried and resuspended in sterile bi-distilled water. **Analysis methods:** Extracted DNA run on 1.2% agarose gel. PCR of 500bp and 1722bp fragments. Sequencing. **Results:** Best methods: cryopreservation, then acetone and 100% ethanol. 75% ethanol, Carnoy's, 2-propanol and silica gel damaged DNA. 2-year stored samples more damaged than fresh samples, but little difference between specimens stored for 2 years or 2 months (at least for silica gel). Sequence accuracy exceeded 96%. **Problems:** Unknown: temperatures of cryopreservation, times of storage (up to 2 years = 1 month to 2 years). Sequence data not provided.
		Direct?	Acetone		2 years	RT					
		Direct?	2-propanol		Up to 2 years	RT					
		Direct?	Carnoy's		Up to 2 years	RT					
		Direct?	Ethanol (75%)		Up to 2 years	RT					
		Direct?	Ethanol (75%)		Up to 2 years	4°C					
		Direct?	Ethanol (100%)		Up to 2 years	RT					
		Direct?	Ethanol (100%)		1 month, 1 year, 2 years	4°C					
		Direct?	Cryopreservation (ultracold freezer)		Up to 2 years						
		Direct?	Cryopreservation (liquid nitrogen)		Up to 2 years						

Table 1. Summary of studies using insects to investigate the effects of preservation materials and storage conditions on DNA. "Direct" = the insects were killed by being placed directly into the preservative solution, "Direct?" = the insects were killed by being placed directly into the preservative solution is implied, "CTAB" = cetyltrimethylammonium bromide, "DEPC" = diethyl pyrocarbonate, "NA" = not available, "RAPD" = randomly amplified polymorphic DNA, "RT" = room temperature, "RT?" = room temperature is implied, "SDS" = sodium dodecyl sulphate, "SSC" = standard saline citrate. The language of the original reference was preserved as much as possible when reporting the methods and results to avoid misinterpretation, hence some inconsistency in terminology and formatting.

As a result of more controlled experiments providing corroborating evidence, it can reliably be said that cryopreservation either in liquid nitrogen or ultracold freezers of fresh specimens is the best method for preserving DNA in insects at least up to 2 years (A'Hara et al. 1998; Carter 2003; Carvalho and Vieira 2000; Mandrioli et al. 2006; Post et al. 1993; Reiss et al. 1995). DNA also remained well preserved in 90%-100% ethanol, particularly when refrigerated, for at least 1 year (Carvalho and Vieira 2000; Mandrioli et al. 2006; Post et al. 1993) and in acetone for between 6 months and 5 years, 4 months (Fukatsu 1999; Mandrioli et al. 2006). Lower concentrations (50-75%) of ethanol were consistently more damaging to DNA than higher concentrations (90-100%) when both were tested (Fukatsu 1999; Mandrioli et al. 2006; Post et al. 1993), although the difference was not always statistically significant depending on the analytical methods employed (Vink et al. 2005). DNA was damaged by storage in Carnoy's solution (Mandrioli et al. 2006; Post et al. 1993; Reiss et al. 1995) and ethyl acetate in as little as 4 months (Carter 2003; Reiss et al. 1995). The effect of storage temperature was also assessed by exposing specimens to the same treatment under different environmental conditions, and several studies formally concluded that storage in organic solvents at lower temperatures was optimal to enhance DNA preservation, although the evidence was contradictory as to whether 4°C (Post et al. 1993) or -20°C was better (Carvalho and Vieira 2000; Vink et al. 2005).

A few studies arrived at conclusions contrary to those mentioned, although shortcomings in research design may account for this. For example, some poor selections of treatments were tested, such as those selected by Gurdebeke and Maelfait (2002) which included 70% ethanol, acetic acid:TE (1:1) and 4% formaldehyde as well as Tayutivutikul et al. (2003) who compared 70% ethanol and rapid hot air drying at 60°C. Although both studies conclude that 70% ethanol is the best treatment to preserve DNA, this is largely due to the fact that the other treatments against which 70% ethanol was compared were prohibitively destructive – formaldehyde has been found to be particularly damaging (Carter 2003), and DNA is known to be damaged by both acidic conditions and heat (Lindahl 1993). When tested in other studies against different alternative treatments, 70% ethanol was not recommended for use, as previously mentioned. Just because 70% ethanol was found to be the best treatment from a selection of poor treatments does not mean it is a good treatment. Based on the overwhelming majority of research results advocating cryopreservation (see above), the somewhat anomalous result by Mtambo et al. (2006) of cryopreserved specimens stored for approximately 2 years not yielding substantially better preserved DNA than the other methods tested (refrigeration drying, refrigeration with silica gel and 70% ethanol) seems unlikely, and this result should be questioned – perhaps shipping at -150°C had some effect, the freezer failed in transit, or something unreported in the specimen history affected DNA preservation.

Some of the more recent studies have started to investigate problems above and beyond selecting preservative solutions and storage temperatures. Dean and Ballard (2001) addressed the issue of the effects of storage with and without naphthalene on DNA, which was found not to have an effect after exposure for up to 2 years. Carter studied the effects of additives such as EDTA, which had previously been suggested to improve DNA preservation (Matsuo et al. 1999), and of changing solutions when hydrated, which had also been suggested as a measure to improve preservation (Fukatsu 1999), however both were found to induce further damage (although it was noted that the effects of changing solutions would be dependent on the specimen:fluid ratio as well as the water content of the specimen (2003: 68)).

In spite of the changes made to the methodology and reporting of results by the biological studies over their predecessors, several problems remain. Some studies used whole, complete insects for individual samples, each digested in the same volume of buffer. In these cases, the starting concentration of DNA will vary across samples due to variations in the size of each specimen, which will affect the comparison of extracted DNA quantification calculations. Some studies used specimens collected and prepared at different times and under different conditions (Post et al. 1993), making direct comparisons between treatments difficult. A continuing problem was the lack of adequate details to enable replication of experiments, in particular the method used to kill insects was often unspecified (although it was occasionally implied to be the direct result of placement in a preservative solution) or omitted, in spite of research indicating that the killing method affected DNA preservation (Dean and Ballard 2001). Additionally, the specimen to fixative fluid ratio, and the length of time passed between DNA extraction and testing as well as the conditions under which DNA extracts were stored were often not reported, all of which could affect the interpretation of reported results. Lastly, much of this work focused on identifying methods to preserve high molecular weight DNA during fieldwork and for the short term (Gurdebeke and Maelfait 2002; Post et al. 1993; Reiss et al. 1995; Rey et al. 2004; Tayutivutikul et al. 2003; Vink et al. 2005) rather than looking to preserve DNA for the long term, which is a subject in urgent need of investigation. It is also unknown if vertebrate tissues would respond to treatments the same way as complete insects did in these studies, and if DNA would be similarly preserved.

In conclusion, the results of most biological studies are largely in agreement with one another, keeping in mind that damage may be due to the failure of a treatment to retard natural processes damaging DNA (for example, incomplete fixation due to poor penetrability into a particular insect), rather than the treatment actively causing damage. The methods and materials used not only for specimen preparation, but also for sample analysis were more thoroughly documented in the biological insect studies, with a few exceptions. As a result, on a cumulative basis the research design of biological studies provides a better model to investigate the effects of

preparation and conservation treatments on DNA, and the experimental results published serve to guide expectations as to the effects of specific chemicals on high molecular weight DNA. However, the methods used tended not to be able to account for all possible variables causing damage, and also were not considered ideal for investigating the effects of preparation and conservation treatments on short strands of DNA.

2.3. Other relevant studies

Although the studies already mentioned are useful for some general conclusions and methodological guidance, much of this research was aimed at preserving high molecular weight DNA or blocking nucleases and other natural degradation processes associated with freshly collected material, and therefore the results obtained may not be directly applicable to short strands of DNA in archaeological or other specimens maintained in collections for any length of time. The studies considered so far have also focused on wet tissue preservation materials and methods, but the majority of collection materials in museums will be maintained in a dry state. Furthermore, the majority of studies previously discussed have been concerned solely with preparation treatments, with the objective of minimising putrefaction, but little thought was given to conservation treatments which might be subsequently employed in the course of collection care and maintenance. Lastly, as it is uncertain if the presence of exoskeletons or other morphological differences would preclude the direct application of the insect studies results to vertebrate tissues, and little could conclusively be ascertained about the effects of treatments from the medical pathology studies, more recent and better designed studies using vertebrate tissues were sought. Although no single study addresses all of these issues, a handful of studies help to fill in knowledge gaps by approaching the study of DNA damage resulting from preparation and conservation treatments using methods, materials or approaches that vary from the studies already mentioned.

One of the first publications specifically addressing the effects of conservation treatments on DNA was written by Brown (1999). Brown acknowledged that few studies had at that time actually tested the effects of conservation treatments on DNA, and he attempted to extrapolate the effects of conservation treatments from existing nucleic acid chemistry knowledge. Brown outlined both general chemical types as well as specific chemicals that were known to cause chemical modifications to DNA, typically *in vitro*, under mild conditions, defined as "at or near physiological pH and ambient temperature" (1999: 136), as well as compounds he felt were probably safe or unsafe to use based upon their presumed effects on DNA. These lists are summarised in Table 2. Although the kinds of DNA damage that might be anticipated as a result of chemical treatment were discussed, namely denaturation, cross-linking, strand breakage and chemical modification, exactly what constituted either a "safe" or "unsafe" chemical was not defined, and no experimental work was undertaken to confirm these predictions.

Unsafe (induce chemical modifications to DNA under mild conditions)	Probably unsafe	Probably safe
Aldehydes	Ammonium hydroxide	Acetone
Formaldehyde	Carbon tetrachloride	Alcohols
Glyoxal	Chloropicrin	Alum
Ninhydrin	Chromic acid	Arsenicals
Alkylating agents	Citric acid	Bendiocarb
Alkyl halides	Dichlorvos	Benzene
Dimethyl sulphate	Ethylene dichloride	Borax
Ammonium derivatives	Glutaraldehyde	Camphor
Hydrazine	Lead salts	Carbolic acid (phenol)
Hydroxylamine	Lindane	Carbon disulphide
Semicarbazide	Mercuric salts	Chloroform
Aromatic nitrogen compounds	Methyl bromide	Chromates
Aromatic amines	Organomercuric salts	Dioxane
Azo-dyes	Organophosphates	Ether
Bisulphites	Paradichlorobenzene	Ethylene oxide
Sodium bisulphite	Pentachlorophenol	Gasoline
Borohydrides	Perchloroethylene	Glycerine
Sodium borohydride	Sodium silicofluoride	Glycerol
Carbodiimides	Sulphur fluoride	Hydrogen cyanide
N,N-dicyclohexylcarbodiimide		Lysol
Halogens		Magnesium carbonate
Bromine		Naphtha
Iodine		Naphthalene
Iodine chloride		Phosphine
Mercurics		Potash
Mercury (II) acetate		Potassium nitrite
Mercury (II) chloride		Potassium phosphate
Nitrites		Propoxur
Nitrous acid		Salt (sodium chloride)
Sodium nitrite		Sodium acetate
N-Nitroso compounds		Sodium bicarbonate
Nitrosourea		Sodium dithionite
Oxidizing agents		Sodium phosphate
Hydrogen peroxide		Turpentine
Osmium tetroxide		
Peracids		
Potassium permanganate		

Table 2. Summary of tables of predictions regarding the effects of chemical treatments on DNA from Brown (1999).

Williams (1999) assessed the effects of a variety of chemical stabilisation treatments on DNA in sections of a fresh otter skin. In addition to an untreated control, skin sections were treated with: alum ($AlNH_4(SO_4)_2$), arsenic trioxide, magnesium carbonate, mercuric chloride, potassium nitrate, sodium borate, sodium chloride, sodium fluosilicate, and formalin. DNA from samples of skin was extracted and visualised on an agarose gel, the purity of the DNA extracted was determined using spectrophotometry, and the extracted DNA was used in PCR amplification. All samples including the control were found to be severely damaged, with alum and potassium nitrate treated samples yielding extracted DNA fragments approximately 500bp in length, the control yielding fragments approximately 200bp in length, and the other treatments yielding fragments between 300 and 500bp long. Amplification by PCR failed for both mitochondrial and nuclear sequences for samples treated with alum, formalin and mercuric chloride. PCR

amplification of nuclear sequences failed for the sample treated with magnesium carbonate and the control. No clear link was found within the data between the preservation of DNA or its successful amplification to either the length of the extracted DNA fragments or their purity.

One of the only studies focusing exclusively on the effects of conservation treatments on DNA tested several fumigants used to kill either pests or fungi for their effects on DNA in samples of freeze dried chicken tissue (Kigawa et al. 2003). Freeze dried mushroom was also used in this test, but different results were obtained which was explained as being due to a difference in cell structure; only the chicken tissue results are considered here because only human and animal tissues are the focus of this research . This study found that methyl bromide (86% by weight)/ethylene oxide (14% by weight), methyl bromide, ethylene oxide, ethylene oxide (15% by weight)/HFC R-134a (85% by weight), propylene oxide and methyl iodide all caused damage to DNA, in the form of strand breakage as visualised on an agarose gel. PCR efficiency was lower for those treatments containing methyl bromide and methyl iodide, however PCR products were successfully obtained and directly sequenced for a 354bp mitochondrial fragment. PCR efficiency was reduced for a longer (1,028bp) mitochondrial fragment by the same treatments as the shorter fragment, as well as by ethylene oxide/HFC R-134a mixed gas, but less so by pure ethylene oxide. It would seem that the source of the damage observed is the HFC R-134a, a refrigerant composed of 1,1,1,2 tetrafluoroethane (DuPont 2002). PCR efficiency of a 1,095bp nuclear DNA fragment was reduced by all treatments found to cause damage, which is expected due to its length and the fact that nuclear DNA exists in lower concentrations than mitochondrial DNA in tissue.

Two main conclusions can be drawn from these studies. Firstly, comparing the predictions made by Brown (1999) about what chemicals were probably safe or unsafe for DNA with the results of the experimental studies by Williams (1999) and Kigawa et al. (2003) demonstrates that predictive models are not entirely satisfactory. Although Brown predicted that methyl bromide, mercuric salts (mercuric chloride), sodium silicofluoride (sodium fluosilicate) and formaldehyde (formalin) were probably unsafe for DNA which is in agreement with the experimental results obtained, ethylene oxide, arsenicals (arsenic trioxide), magnesium carbonate, sodium chloride and alum (although which form of alum was unspecified) were predicted to be safe, but all were found to be damaging. Secondly, the relationship between treatments and PCR amplification is also not necessarily straightforward, as extracted DNA samples that appear similarly damaged may PCR amplify to different degrees. Further experimental work is required to better characterise the reactivity of DNA with the most commonly used preparation and conservation treatments to better gauge the utility of existing collections and to devise conservation strategies to improve biochemical preservation within the collection environment.

Although much as been done by the biochemical profession to develop methods enabling the increased use of archival collections from the past in research, it is now time for more conservators to undertake research in order to initiate measures to improve DNA preservation in future collections. This requires a fundamental shift in research ideology as well as new methods to identify the influence of the different variables affecting short strands of DNA, such as specific chemical species, exposure time to chemicals, temperature, etc., with the intention of revising conservation approaches to biological material in collections to actively preserve DNA.

Chapter 3. Survey of documented preparation and conservation treatments used in the past

Following a review of the existing literature addressing the effects of preparation and conservation treatments on DNA, gaining a better understanding of the history of treatments used in collections was necessary. Therefore, a literature review across all collection types of any possible treatments administered to both human and animal hard and soft tissues was undertaken, and a database of treatment references was compiled. Although the reasons for a broad-based approach will be discussed in detail below, in general this was done to take into consideration the transient nature of collections in the past as well as the variety of materials and methods used on these specimens by practitioners in the different subdisciplines involved.

3.1. Scope of the survey

Approaches to both human and animal remains were documented for several reasons. Although human remains are considered of greater importance over animal remains in some collections, for the purposes of this research, they are considered identical in a materials sense (Reid 1994). It can therefore be assumed that preparation and conservation treatments mentioned in the literature for animal material could also have been used for human material (Cooke pers. comm. 2003) and vice versa (Howie 1986b). Furthermore, in some exceptional cases, human remains have been used as source material in objects traditionally derived from animal materials such as book bindings (Schmitzer 1986), jewellery and talismans (Hough 1908), masks (Joyce 1926; Nicklin 1974, 1979, 1983), and musical instruments (Baby 1961; Libin 1977-1978; Mainfort 1988; von Winning 1959). As methods specific to the production or care of such objects are often not provided, they must be deduced from references regarding objects made from the more typical animal sources. In terms of DNA research, it could be argued that animal remains are preferred for use over human remains, as their use enables easier contamination control and is generally subject to fewer ethical restrictions, which may result in their being made more readily available for destructive sampling. Animal remains may become increasingly more important serving as a proxy for human remains (Hurles et al. 2003; Matisoo-Smith and Robins 2004), an approach taken in this study to look at the effects of mummification.

Both hard and soft tissues were of interest in this study, as various tissues have been used in DNA studies. Also, specimens could be subject to further processing over time due to changing collection requirements or deterioration making the original preparation unsuited for its initial purpose but still useful in other ways (i.e. cadavers yielding soft tissue and/or bone specimens). As a result, the full range of treatments administered in order to prepare fresh tissues as well as maintain dry and wet specimens was included in the literature review.

Both preparation and conservation treatments were documented in this study. Early on there was little distinction between treatment types, but over time they developed into different trades with their own methods and materials, and both continued to be applied to human and animal remains. A brief history of these professions sheds light on the range of treatments carried out on tissue collections over time.

Although the terms "preparator" and "conservator" have quite specific definitions and associated duties in different places today, this was not always the case and the roles and responsibilities embodied in these terms evolved over time. While a bias can be seen in the literature towards the early use of the term preparator in Canada and the United States and conservator in Britain, this was by no means strictly adhered to, and occasionally the same individuals referred to as either a conservator or preparator were also called by the title curator (Anonymous 1870). Some of the earliest published references in the field refer to the work of the many well-known conservators employed from the turn of the 19th century at the Hunterian Museum, Royal College of Surgeons, such as William Clift, Richard Owen, William Henry Flower and John Quekett, whose various duties included dissection (Home 1801), preparing soft tissue specimens (Flower 1860-1862; Home 1809; Owen 1834), making casts (Anonymous 1864), producing microscopy and histology preparations (Anonymous 1862-1863), as well as inspecting and identifying animal bones (Whidbey and Clift 1815-1830) and fossil material (Flower 1872-1873; Huxley 1862-1863). In the 1860s, preparators begin to be mentioned in the literature undertaking a number of similar tasks as well as additional duties. In 1905, preparators at the American Museum of Natural History were "completely overhauling and cataloguing" the collection (H. F. O. 1905a: 188) and "restoring" and mounting palaeontological specimens (H. F. O. 1905b: 375). In his obituary in 1876, Mr. Julius Stoerzer of the National Museum at Washington was referred to as a "scientific taxidermist" and "an artist as well as a preparator" (Anonymous 1876: 507), and by 1917 the term preparator was preferred over that of taxidermist (Nutting 1917: 15). By the mid-20th century, the distinction between conservators and preparators was being made with both terms being used to define responsibilities or staff within the museum context (Anonymous 1964, 1971; Parkhurst 1955; Ruggles 1973). At this time, preparators continued to operate within biological, zoological, geological, palaeontological, anatomical and medical museums with little change to their responsibilities, but preparators in archaeological, anthropological, ethnographic and art museums tended to be relegated to mount making and assisting with object transport and setting up exhibitions rather than treating objects. Indeed, traditional preparators began to get a bad reputation, with taxidermists being referred to as "a small group of men who were more or less secretive and jealous of their methods" (Moyer 1953: 6), an assertion repeated by Madsen, who also stated that preparators lacked specialised skills, having been trained only through an

apprentice system (1973: 225). By the 1990s, according to Knell and Collins there was little room for preparators in museums, as "[t]he conservator aims to do the minimum necessary to ensure that the fossil or mineral remains of scientific or educational use. Stepping beyond these bounds he becomes a preparator, restorer or faker" (1992: 64). Conservators introduced various codes of ethics and codes of practice (American Institute for Conservation of Historic & Artistic Works 1994; Canadian Association for Conservation of Cultural Property and the Canadian Association of Professional Conservators 2000; European Confederation of Conservator-Restorers Organisations 2002) to correct the wrongs of preparators who were accused of not having a formal education as a part of their training, a lack of documentation and transparency in reporting chemical treatments, a tendency to over embellish, as well as the selection of extreme or permanent treatment materials.

With this history in mind, for the purposes of this study "preparation treatments" have been defined as initial treatments undertaken to render material suitable for exhibition or admission to a collection, typically of palaeontological or freshly collected biological material. Preparation treatments were often undertaken by collectors, amateurs or were passed down through preparators within individual institutions. Preparation treatments may differ from conservation treatments in the materials used (often quite harsh or proprietary), the quantity of materials used (often excessive), or their purpose (often aesthetic). "Conservation treatments" are defined as treatments undertaken with the intention of preservation, and are designed using materials and methods in conformance with the conservation principles of minimal intervention, reversibility (whenever possible), preservation of maximum information, maintenance of the state of preservation when recovered or acquisitioned, full documentation and disclosure of materials and methods used. Such treatments may be administered in the field or within collections at any time, and are generally undertaken by trained conservators. The distinction between conservation and preparation treatments is not always clear, and many specimens may have been subjected to both over the course of their treatment history.

As the sciences became increasingly specialised throughout the 19th century, so did museums (Asma 2001), and today both human and animal remains, even archaeological remains, are held in a variety of different kinds of collections, including but not limited to: art, archaeological, anthropological, ethnographic, biological, zoological, palaeontological, geological, anatomical and medical collections. Historically, many of these types of materials may have been maintained together as "natural history" collections (Bateman 1975; Reid 1994), a term avoided here due to its all-encompassing nature. Material type may not necessarily dictate the kind of collection in which an object will ultimately be housed. Many well-known archaeological specimens are held within other types of museum collections, such as Reisner's ancient Egyptian collections at the Boston Museum of Fine Art. Freshly procured reference collection

specimens similarly may be maintained for comparison within archaeological collections. Therefore, when thinking of the treatment history of human remains in general, it may not be accurate to consider them only within the framework of the type of collection they currently comprise.

Private cabinets of curiosity and smaller collections moved between museums (Kohlstedt 1988) and over time have tended to be incorporated into larger museums (Alberti 2002; Causey and Trimble 2005). In fact the movement of collections was so great at the end of the 19th century and the beginning of the 20th century it was the subject of a 147 page catalogue, *Where is the - Collection?: An account of the various natural history collections which, between 1880 and 1939 have come under the notice of the compiler* (Sherborn 1940). This is significant because different collection types have tended to use different materials and methods for collection preparation and conservation. Due to the overlapping interests and influences of the different academic fields concerned with human and animal remains, it was considered inappropriate to focus on the methods and materials used by one profession alone, as that was probably not a realistic representation of what has happened to collections material.

3.2. Treatments documented in the past

The materials and methods employed by different professions to care for human and animal remains greatly depend upon whether or not the material when collected was fresh or long deceased (e.g. buried, mummified, fossilised, etc.), if the material was maintained as wet, soft tissue or dry skeletal remains, and the development of conservation ideals within a particular field of study. Other variables apply to material collected in the field, such as the specimen's stability when recovered and transport to the final storage location. Additionally, the intended purpose or use of the material may affect its treatment, such as whether it was to be used as a teaching, reference or research collection, and whether or not it was to be exhibited.

Several studies have surveyed treatments in the past, but they have tended to focus on either a particular kind of collection, such as spirit collections (Stoddart 1989) and fossils (Howie 1984, 1986a) or a specific treatment type such as adhesives and consolidants (Elder et al. 1997; Johnson 1994; Koob 1981, 1984; Shelton and Chaney 1994; Shelton and Johnson 1995), pest control (Goldberg 1996; Hawks 2001; Pereira and Hammond 2001; Pereira and Wolf 2001) and acid preparation (Lindsay 1987; Toombs and Rixon 1959). Although these studies were useful starting points in amassing the relevant literature, no studies to date attempted to survey fully the range of treatments applied to human and animal hard and soft tissues. It was considered inappropriate to follow the precedent of these earlier reviews and to test only materials reportedly used for a particular treatment or collection type, because it would ignore the complex history of many specimens. Specimens have been moved between collections, have

gone through different phases of processing and may have been treated repeatedly over time for different reasons using a variety of chemicals. It was considered important to consider the cumulative effects of the treatment history of specimens.

To assess the full range of materials and methods used in preparation and conservation treatments for human and animal remains in the past, a literature review across all relevant disciplines was undertaken. Only English language references were included in this study, as this represented a substantial and varied, yet discrete body of literature of which a representative sample was accessible. Furthermore, it was quickly realised that even within the English language references, understanding and accurately replicating instructions for treatments as published was not necessarily straightforward and error was easily introduced due to regional and professional language preferences, changes in terminology over time and inconsistent uses of nomenclature; including another body of literature without total command of that language was deemed both unnecessary and ill advised. References were primarily collected from the period after 1880, as it was thought this was the timeframe from which many existing collections would date, and it is when the majority of references began to be published. Approximately 400 publications were surveyed (for a complete list of references surveyed, see Appendix A). For every treatment mentioned, each chemical used in any given treatment was recorded as a separate database entry to allow for the comparison of chemical use across treatment types. In addition to the chemical and any instructions for use, the following information was also recorded for each chemical reference when provided: concentration, temperature, solvent, exposure time, purpose of treatment, trade names and common names, collection type and tissue treated. Out of a total of just under 3000 chemical treatment database entries, approximately 475 individual chemicals were identified for use (see Table 3 for a full list), of which 38 were mentioned only by trade name, the active ingredient of which could not be identified (these chemicals are listed in quotes, e.g. "1001 cleaner").

Materials historically used on collection objects
"1001 cleaner"
"Blue Cloud"
"Brillac"
"Bronze powder"
"Buhac"
"Carpenter's glue"
"Casco Contact clear instant adhesive"
"Catalin"
"Celastic"
"Cement"
"Cerric Bronzing Medium D448 with Cerric Thinning Medium T6"
"Cetrimide"
"Chlorosol"
"Clear plastic spray"
"Cuprinol Clear #21"
"Demovit"

Materials historically used on collection objects
"Distillate"
"Egyptian Cement"
"Hydroxide"
"Lakeside 70"
"Marine glue"
"Metallic X" (glue)
"Parmetol K40"
"Perpetuin fixative"
"Pif paf"
"Planatol"
"Plastic spray"
"Quentglaze Accelerator 405/1"
"Quentglaze Sealer 531/1"
"Quentglaze"
"Resin"
"RTV rubber compound"
"Savlon"

Materials historically used on collection objects
"Skin paste"
"Texicryl 13-002"
"Veneno"
"Vinamold"
"Xylamon" (contains lindane?)
(2R,6aS,12aS)-1,2,12,12a-tetrahydro-8,9-dimethoxy-2-(1-methylethenyl)[1]benzopyrano[3,4-b]furo[2,3-h][1]benzopyran-6(6aH)-one [aka Rotenone]
(5Z,7E)-(3S)-9,10-seco-5,7,10(19)-cholestatrien-3-ol
1,1,1-trichloro-2,2-bis[p-methoxyphenyl]-ethane
1,1,1-trichloro-2-methyl-2-propanol
1,2,3,4,10,10-hexachloro-6,7-epoxy-1,4,4a,5,6,7,8,8a-octahydro-endo-1,4-exo-5,8-dimethanonaphthalene
1,2,4,5-tetramethylbenzene
1,2,4-trichlorobenzene
1,2-di(ethoxycarbonyl)ethyl O,O-dimethyl phosphorodithioate
1-[(6-chloro-3-pyridinyl)methyl]-N-nitro-2-imidazolidinimine
1-butanol
1-heptadecanecarboxylic acid [aka stearic acid]
1-methylethyl (2E)-3-[[(ethylamino)methoxyphosphinothioyl]oxy]-2-butenoate
1-methylethyl (2E,4E)-11-methoxy-3,7,11-trimethyl-2,4-dodecadienoate
1-naphthalenol methylcarbamate
1-naphthyl methylcarbamate
1α,2α,3β,4α,5α,6β-hexachlorocyclohexane [aka Lindane]
2-(8-Heptadecenyl)-4,5-dihydro-1H-imidazole-1-ethanol
2,2a,3,3,4,7-hexachlorodecahydro-1,2,4-methenocyclopenta[c,d]pentalene-5-carboxaldehyde
2,2-dimethyl-1,3-benzodioxol-4-yl methylcarbamate
2-[2-(4-chlorophenyl)-2-phenylacetyl]indan-1,3-dione
2-camphonone or 2-boranone
2-ethoxyethyl acetate
2-hydroxybenzoic acid
2-isopropoxyphenyl methylcarbamate
3-(α-acetonylbenzyl)-4-hydroxycoumarin [aka Warfarin]
3,4,5,6,7,8,8a-heptachlorodicyclopentadiene
3-[1-(4-chlorophenyl)-3-oxobutyl]-4-hydroxy-2H-1-benzopyran-2-one
3-[3-(4'-Bromo[1,1'-biphenyl]-4-yl)-3-hydroxy-1-phenylpropyl]-4-hydroxy-2H-1-benzopyran-2-one
4-chloro-3,5-demethylphenol
4-hydroxy-3-(1,2,3,4-tetrahydro-1-naphthalenyl)-2H-1-benzopyran-2-one
4-hydroxy-4-methyl-2-pentanone [aka diacetone alcohol]
4-hydroxynitrobenzene
5-norbornene-2,3-dimethanol, 1,4,5,6,7,7-hexachloro-,cyclic sulfite, exo-; 0.013% in methanol
Abrus seeds
Absinthium
Acetic acid
Acetone
Acrylic
Acrylic copolymer
Acrylic dispersion/emulsion
Agar
Albumin
Alcohol
Aldrin
Alkoxy silanes
Alkyd resins
Aluminium ammonium sulfate
Aluminium phosphide
Aluminium potassium sulfate
Aluminium potassium sulfate, dodecahydrate [aka alum]

Materials historically used on collection objects
Aluminium sulfate
Ammonium chloride
Ammonium citrate
Ammonium hydroxide
Amyl acetate
Animal glue
Antimony
Antimony trioxide
Argon
Arsenic
Asbestos
Atmospheric atomic oxygen source
Axle grease
Bacon grease
Bakelite
Balm [unspecified]
Balsalm [unspecified]
Bark
Beef fat
Beeswax
Benzalkonium chloride
Benzene
Benzine
Benzoic acid
Biocides
Bis(8-hydroxyquinolinium) sulfate
Bitumen
Borax or Boric acid [aka disodium tetraborate decahydrate]
Bouin's fluid
Brains
Bran
Brandy
Bread
Butyl acetate
Butyl methacrylate
Butylated hydroxytoluene
Cacao blossoms
Calcium carbonate
Calcium chloride
Calcium hydroxide
Calcium hypochlorite
Calcium oxide [aka lime]
Calcium phosphate
Calcium sulfate hemihydrate [aka plaster of Paris]
Camphor
Canada balsam
Carbolic acid [aka phenol]
Carbolic disinfecting powder
Carbon dioxide
Carbon disulfide
Carbon monoxide
Carbon tetrachloride
Carnauba wax
Carnoy's fluid
Casein
Casein glue
Casein water paint
Castor oil
Catechols
Catechu
Cedarwood oil
Cellulose

Materials historically used on collection objects
Cellulose acetate
Cellulose nitrate
Chaff
Charcoal
Chloral hydrate
Chlorinated hexahydromethanoindene
Chlorine
Chloroform
Chloropicrin
Chromic acid
Chromium potassium sulphate
cis or trans 1-(3-chloroallyl)-3,5,7-triaza-1-azoniaadamantane chloride
Citric acid
Clay
Cloth
Copal
Cornmeal
Cotton
Cottonseed oil
Creosote (beechwood)
Cresylic acid
Cryogenesis (dry ice)
Cupric 8-quinolinoxide
Cyanide
Cyano(4-fluoro-3-phenoxyphenyl)methyl 3-(2,2-dichloroethenyl)-2,2-dimethylcyclopropanecarboxylate
Cyanoacrylates
Dermestids
Detergent
Dextrine
Diammonium citrate
Diatomaceous earth
Dibasic sodium phosphate
Dichlorodifluoromethane
Dichloro-diphenyl-trichloroethane
Dichlorvos or 2,2 dichlorovinyl dimethyl phosphate
Diethyl [(dimethoxyphosphinothioyl)thio]butanedioate
Diethylene dioxide
Dimethyl formamide
Dry ice
Dry pigments
Dubbin
Egg yolk
Enzymes (including: Axion, Biz, chymopapain, collegenase, Neutrase, pancreatin, papain, pepsin, trypsin and unknown varieties)
Epoxies
Ethanol
Ethanolamine thioglycollate
Ether
Ethyl acetate
Ethyl methyl ketone
Ethylacetate/nitrocellulose resin
Ethylcellulose
Ethylene diaminetetracetic acid (and its sodium salts)
Ethylene dibromide or 1,2 dibromoethane
Ethylene dichloride or 1,2 dichloroethane
Ethylene dioxide
Ethylene oxide or dimethylene oxide
Ethylhydroxyethyl cellulose/polyethylene glycol
Excelsior
Fat
Fish glue

Materials historically used on collection objects
Flax
Flour
Flour paste
Formalin
Formic acid
Fungicide
Gamma radiation
Gasoline
Glue
Glycerin
Grass
Gum [unspecified]
Gum arabic
Gum dammar
Gum tragacanth
Gutta-percha
Hay
Hexamine
Hexane
Hops
Hydrochloric acid
Hydrofluoric acid
Hydrogen peroxide
Hydrogen phosphide
Hypo-acetine
Industrial methylated spirit
Insects
Iodine
Isopropanol
Jute
Kaolin
Kerosene
Kettle descaler
Lacquer
Lactic acid
Lanolin
Lantern fuel (naptha or gasoline before 1915)
Lard oil
Latex paint
Lauryl pentachlorophenate
Lead
Lead arsenate
Leaves
Linseed oil
Lithium carbonate
Lysol
Magnesium carbonate
Magnesium oxide
Magnesium silicate
Magnesium sulfate
Marmite
Mastic
Mealworms
Mercury
Methanol
Methyl amyl alcohol
Methyl bromide
Methyl methacrylate
Methylcellulose
Methylene chloride
Methylmethacrylate/ethylacrylate resin
Microwave

Materials historically used on collection objects
Modelling clay
Monobasic sodium phosphate
Musca larvae
Musk
N-[[[3,5-dichloro-4-(1,1,2,2-tetrafluoroethoxy)phenyl]amino]carbonyl]-2,6-difluorobenzamide
Naphthalene
Neat's foot oil
Nicotine or 3-(1-methyl-2 pyrrolidyl) pyridine
Nitric acid
O,O,O',O'-Tetraethyl S,S'-methylene bis (phosphorodithioate)
O,O-diethyl O-(3,5,6-trichloro-2-pyridinyl) phosphorothioate
O,O-diethyl-O-(p-nitrophenyl)thionophosphate
O,O-dimethyl O-4-nitro-m-tolyl phosphorothioate
Oil [unspecified]
Oil of aniseed
Oil of sassafras
Oil paint
Oil St. Rocco
Ojie bakut
Olive oil
Orthene
Ortho-dichlorobenzene
Osmic acid
Oxalic acid
Paint
Palm oil
Paper pulp
Papier mache
Paradichlorobenzene or 1,2 dichlorobenzene
Paraffin
Paraffin wax
Paraformaldehyde
Paraldehyde
p-diisobutyl phenoxyethoxyethyl dimethyl benzylammonium chloride [aka benzethonium chloride]
Pentachlorophenol
Pentanedial [aka glutaraldehyde]
Pepper
Peroxides
Petroleum ether
Petroleum jelly
Phenoxetols
Phosphorothioic acid O,O-diethyl O-[6-methyl-2-(1-methylethyl)-4-pyrimidinyl] ester
p-hydroxybenzoic acid
Picric acid
Pigment
Pine oil
Pitch
Plastic (clear)
Plastic [unspecified]
Platinic chloride
Poly(vinyl) acetal
Poly(vinyl) acetal resin
Poly(vinyl) acetate resin
Poly(vinyl) acetate emulsion
Poly(vinyl) acetate/poly(vinyl) alcohol emulsion
Poly(vinyl) acetyl resins
Poly(vinyl) alcohol
Poly(vinyl) butyral

Materials historically used on collection objects
Poly(vinyl) butyral resin
Poly(vinyl) chloride-acetate
Polyamides
Polybutyl methacrylate
Polyesters
Polyethylene glycol
Polylvinyledene chloride emulsion
Polymethyl methacrylate
Polystyrene
Polyvinylidene chloride
Potassium acetate
Potassium carbonate [aka potash]
Potassium chlorate
Potassium dichromate
Potassium hydroxide [aka caustic potash]
Potassium nitrate [aka saltpeter]
Potassium permanganate
Potassium sulfate
Propylene glycol
Pyrethrins and pyrethroids
Pyridine
Pyrogallols
Radiography
Rags
Rattan cane
Resin [unspecified]
Rhigoline
Rice hull ash
Rosin
Rubber
Rubber cement
Rum
Sago palm leaves
Salt
Sand
Sawdust
Scale remover
Sea animals
Seaweed gum
Shellac
Shoe polish
Silane
Silica gel
Silicon carbide
Silicone
Silicone esters
Silicone fluid
Silicone resins
Size
Smoke
Sodium acetate
Sodium aluminium fluorosilicate
Sodium bicarbonate
Sodium borate
Sodium chloride [aka salt]
Sodium carbonate [aka soda, sal soda, washing soda]
Sodium disilicate
Sodium dithionite
Sodium fluoride
Sodium fluorosilicate
Sodium fluosilicate
Sodium hexametaphosphate

Materials historically used on collection objects
Sodium hydrosulphite
Sodium hydroxide
Sodium hypochlorite
Sodium hyposulfite
Sodium orthophenyl phenol
Sodium orthophosphate
Sodium perborate
Sodium perborate tetrahydrate
Sodium peroxide
Sodium phosphate
Sodium silicate
Sodium sulfate
Sodium sulfide
Sodium thiosulphate
Sodium thiosulphate (hypo)
Sodium-p-toluene-sulphachloramine, chlorazine
Soil
Solder (liquid)
Soluble nylon
Sorbitol
Spermaceti
Sphagnum moss
Starch
Stearic acid
Stearine
Straw
Strychnine or strychnidin-10-one
Styrene acrylate
Sugar
Sulfur
Sulfur dioxide
Sulfuric acid
Sulfuryl fluoride or sulphur difluoride dioxide
Sulphonated neats-foot oil
Sumac
Sun
Talc
Tallow
Tanacetum

Materials historically used on collection objects
Tannic acid
Tanning fluid
Tannins [unspecified]
Tar [unspecified]
Tartaric acid
Tertiary butyl alcohol
Tetrachloroethylene
Tetrahydro-5,5-dimethyl-2(1H)-pyrimidinone [3-[4-(trifluoromethyl)phenyl]-1-[2-[4-(trifluoromethyl)phenyl]ethenyl]-2-propenylidene]hydrazone
Thioglycollic acid
Thymol
Toluene
Tow
Trichloracetic acid
Trichloroethane or 1,1,1-trichloroethane
Trichloroethylene
Trisodium phosphate
Trisodium phosphate dodecahydrate
Turpentine
Urea
Varnish
Vegetable fibre
Vinegar
Vinyl acetate
Vinyl acetate/olefin copolymer
Vitamin C
Water
Water softener
Wax [unspecified]
Wax-resin mixtures
White gasoline
White glue
White spirit, mineral spirits [naptha or Stoddard's solvent]
Whiting
Xylene
Zinc
Zinc chloride

Table 3. List of materials identified in the literature review historically used in preparation and conservation treatments.

Identifying the various common and trade names used for each of the chemicals whenever possible was necessary for this research in order to identify accurately and consistently the active ingredients used in treatments. For the purposes of this research, a currently accepted chemical or common name is used for ease of reference, but the Chemical Abstracts Service (CAS) registry number is also provided when available, as it is a unique identifier, specifically referring to a single chemical species. Each of the chemicals identified in the literature review had been referred to by several names. Common names and trade names were most numerous, but due to changes in accepted chemical nomenclature, chemical names were also diverse. The common names, trade names and chemical names of the 44 chemicals tested in the screening experiment are listed in Table 4 (the criteria used to select these treatments are outlined in section 4.1.1). This list is not intended as an exhaustive inventory of terms used to identify the chemicals used, but it does serve to illustrate the variety of terms employed in the past. In

addition to the sheer number of synonyms used, terms were also occasionally misused. To represent faithfully the literary record of treatments, misused terms were included in Table 4, as treatments may then have been replicated using the incorrect material(s); the erroneous use of terms was identified when recognised. A clear understanding of the etymology of treatments is, therefore, essential to understanding the past use of chemicals in preparation and conservation treatments.

Materials used [CAS number]	Common and trade names
Acetic acid [64-19-7]	4-02-00-00094 (Beilstein Handbook Reference); Acetasol; Acetic acid (natural); Acetic acid [JAN]; Acetic acid solution, not less than 50% but more than 80% acid, by mass [UN2790] [Corrosive]; Acetic acid solution, with more than 10% and less than 50% acid, by mass [UN2790] [Corrosive]; Acetic acid, glacial; Acetic acid, glacial or acetic acid solution, >80% acid, by mass [UN2789] [Corrosive]; Acetic acid, of a concentration of more than 10 per cent, by weight, of acetic acid; Acetic acid, water solutions; Acetone carboxylic acid (B.P.); *Acide acetique* [French]; *Acide acetique glacial* [French]; *Acido acetico* [Italian]; Aci-Jel; AI3-02394; *Azijnzuur* [Dutch]; BRN 0506007; Caswell No. 003; CCRIS 5952; EINECS 200-580-7; EPA Pesticide Chemical Code 044001; *Essigsaeure* [German]; Ethanoic acid; Ethylic acid; FEMA No. 2006; FEMA Number 2006; Glacial acetic acid; HSDB 40; *Kyselina octova* [Czech]; Methane carboxylic acid; Methanecarboxylic acid; NSC 132953; *Octowy kwas* [Polish]; Otic Domeboro; Otic Tridesilon; Pyroligneous acid; Shotgun; Spirit acid (concentrated acetic acid obtained by distillation of 12% vinegar); TCLP extraction fluid 2; UN2789; UN2790; Vinegar; Vinegar acid
Acetone [67-64-1]	2-Propanone; *Aceton* [German, Dutch, Polish]; Acetone; Acetone (natural); Acetone [UN1090] [Flammable liquid]; AI3-01238; β-Ketone propane; β-Ketopropane; beta-Ketopropane; Caswell No. 004; CCRIS 5953; Chevron acetone; Dimethyl ketone; Dimethylformaldehyde; Dimethyl formaldehyde; Dimethylketal; EINECS 200-662-2; EPA Pesticide Chemical Code 004101; FEMA No. 3326; HSDB 41; Ketone propane; Ketone, dimethyl; Methylacetal; Methyl ketone; Methyl ketone; NSC 135802; Propanone; Pyroacetic acid; Pyroacetic ether; RCRA waste no. U002; RCRA waste number U002; UN1090
Acrylic dispersion/emulsion	Acrysol; Acrysol WS-24; Lascaux; Primal AC 33; Primal AC 634; Primal AC-61; Primal B60A; Primal WS 12; Primal WS24; Primal WS50; Revacryl 452; Revacryl 453; Rhoplex AC-33; Rhoplex B60A; Rhoplex WS24
Aluminium potassium sulfate (alum) [10043-67-1 (anhydrous); 7784-24-9 (dodecahydrate)]	Alaun; Alum; Alum potassium; Alum, N.F.; Alum, potassium; Alum, Potassium [USAN]; Alum, potassium anhydrous; Alum, potassium, dodecahydrate; Aluminium potassium bis(sulphate); Aluminum potassium alum; Aluminum potassium disulfate; Aluminum potassium disulfate dodecahydrate; Aluminum potassium sulfate; Aluminum potassium sulfate (1:1:2) dodecahydrate; Aluminum potassium sulfate (AlK(SO4)2); Aluminum potassium sulfate (AlK(SO4)2), dodecahydrate; Aluminum potassium sulfate (JP14); Aluminum potassium sulfate (KAl(SO4)2); Aluminum potassium sulfate dodecahydrate; Aluminum potassium sulfate, alum; Aluminum potassium sulfate, anhydrous; Aluminum potassium sulfate, dodecahydrate; Burnt alum; Burnt potassium alum; C13190; CCRIS 6842; Common alum; Dialuminum dipotassium sulfate; EINECS 233-141-3; Exsiccated alum; HSDB 2685; Kalinite; Potash alum; Potash alum dodecahydrate; Potassium alum; Potassium alum dodecahydrate; Potassium aluminum alum; Potassium aluminum disulfate dodecahydrate; Potassium aluminum sulfate; Potassium aluminum sulfate (1:1:2); Potassium aluminum sulfate dodecahydrate; Potassium aluminum sulfate dodecahydrate (KAl(SO4)2.12H2O); Sulfuric acid, aluminum potassium salt (2:1:1); Sulfuric acid, aluminum potassium salt (2:1:1), dodecahydrate; Tai-Ace K 150; Tai-Ace K 20; White alum; [erroneously aluminium sulfate or papermaker's alum]
Ammonium hydroxide [1336-21-6]	Amex; Ammonia (ammonium hydroxide) 28% by weight or more NH3; Ammonia aqueous; Ammonia solution; Ammonia solution, relative density <0.880 at 15 degree C in water, with >50% ammonia [UN3318] [Nonflammable gas, Poison gas]; Ammonia solution, strong; Ammonia solutions, relative density <0.880 at 15 C in water, with >35% but not >50% ammonia [UN2073] [Nonflammable gas]; Ammonia solutions, relative density between 0.880 and 0.957 at 15 C in water, with >10% but not >35% ammonia [UN2672] [Corrosive]; Ammonia water; Ammonia water 29%; Ammonia, aqua; Ammonia, aqueous; Ammonia, aqueous solution; Ammonia, monohydrate; Ammonia-15N; Ammonium aqueous (28% or less NH3); Ammonium hydroxide; Ammonium hydroxide ((NH4)(OH)); Ammonium hydroxide ((NH4)(OH)); Ammonium hydroxide (28% or less ammonia); Ammonium hydroxide, redistilled; Ammonium, aqueous; Aqua ammonia; Aquammonia; Aqueous ammonia; Caswell No. 044; EINECS 215-647-6; EPA Pesticide Chemical Code 005301; Household ammonia; HSDB 5125; Hydroxyammonium; Spirit of hartshorn; SX 1; SX 1 (ammonia water); UN2073; UN2672; UN3318

Materials used [CAS number]	Common and trade names
Amyl acetate [628-63-7]	1-Pentanol acetate; 1-Pentyl acetate; 4-02-00-00152 (Beilstein Handbook Reference); *Acetate d'amyle* [French]; Acetic acid, amyl ester; Acetic acid, pentyl ester; AI3-02729; Amyl acetate; Amyl acetate (commercial); Amyl acetate (mixed isomers); Amyl acetate, n-; Amyl acetates [UN1104] [Flammable liquid]; Amyl acetic ester; Amyl acetic ether; Amylacetic ester; *Amylazetat* [German]; *Amylester kyseliny octove* [Czech]; Banana oil; Birnenoel; BRN 1744753; Caswell No. 049A; Chlordantoin; Dymon SWH Wasp & Hornet Spray; EINECS 211-047-3; EPA Pesticide Chemical Code 000169; Holiday Pet Repellent; Holiday Repellent Dust; HSDB 5126; n-Amyl Acetate; n-Amyl acetate, normal; n-Amyl acetate, normal (natural); n-pentyl acetate; n-Pentyl ethanoate; NSC 7923; *Octan amylu* [Polish]; Pear oil; pentacetate; Pent-acetate; Pent-acetate 28; Pentyl acetate; Pentyl acetate, all isomers; Pentyl ester of acetic acid; Prim-amyl acetate; Primary amyl acetate; UN1104; 1-pentyl acetate
Arsenic (arsenic trioxide) [1327-53-3]	*Acide arsenieux* [French]; AI3-01163; *Anhydride arsenieux* [French]; *Arseni trioxydum*; Arsenic (III) oxide; Arsenic (III) trioxide; Arsenic (white); *Arsenic blanc* [French]; Arsenic oxide; Arsenic oxide (3); Arsenic oxide (As2O3); Arsenic sesquioxide; Arsenic sesquioxide (As2O3); Arsenic trioxide; Arsenic trioxide [JAN]; Arsenic trioxide [UN1561] [Poison]; Arsenic(III) oxide; *Arsenicum album*; *Arsenigen saure* [German]; Arsenious acid; Arsenious Acid Anhydride; Arsenious oxide; Arsenious oxide, 99.999%; Arsenious trioxide; Arsenite; Arsenolite; Arsenous acid; Arsenous acid anhydride; Arsenous anhydride; Arsenous oxide; Arsenous oxide [ISO]; Arsenous oxide anhydride; Arsenous trioxide; Arsentrioxide; Arsodent; Caswell No. 059; CCRIS 5455; Claudelite; Claudetite; Crude arsenic; Diarsenic oxide; Diarsenic trioxide; Diarsonic trioxide; EINECS 215-481-4; EPA Pesticide Chemical Code 007001; HSDB 419; *Oxyde Arsenieux* [ISO-French]; RCRA waste no. P012; RCRA waste number P012; Trisenox; UN 1561; UN1561; White arsenic
Benzene [71-43-2]	(6)Annulene; AI3-00808; *Benzeen* [Dutch]; *Benzen* [Polish]; Benzene; Benzene (including benzene from gasoline); Benzene [UN1114] [Flammable liquid]; Benzene, pure; Benzin; Benzin (Obs.); Benzine; Benzine (Obs.); Benzol; *Benzol* [German]; Benzol 90; Benzol diluent; Benzole; *Benzole* [French]; Benzolene; *Benzolo* [Italian]; Bicarburet of hydrogen; Carbon oil; Caswell No. 077; CCRIS 70; Coal naphtha; Cyclohexatriene; EINECS 200-753-7; EPA Pesticide Chemical Code 008801; *Fenzen* [Czech]; HSDB 35; Mineral naphtha; Motor benzol; NCI-C55276; Nitration benzene; NSC 67315; Phene; Phenyl hydride; Phenylhydride; Polystream; Pyrobenzol; Pyrobenzole; RCRA waste no. U109; RCRA waste number U019; UN 1114; UN1114
Carbon tetrachloride [56-23-5]	AI3-04705; Benzinoform; Carbon chloride; Carbon chloride (CCl4); Carbon tet; Carbon tetrachloride; Carbon tetrachloride [BSI:ISO]; Carbon tetrachloride [UN1846] [Poison]; Carbon tetrachloride; Carbona; Caswell No. 164; CC m0; CCRIS 123; *Chlorid uhlicity* [Czech]; *Czterochlorek wegla* [Polish]; EINECS 200-262-8; ENT 27164; ENT 4,705; EPA Pesticide Chemical Code 016501; Fasciolin; Flukoids; Freon 10; Freon® 10; Halon 1040; Halon® 104; HSDB 53; Methane tetrachloride; Methane, tetrachloro-; Necatorina; Necatorine; NSC 97063; Perchloromethane; Phenoxin; Pyrex; R 10; R 10 (Refrigerant); RCRA waste no. U211; RCRA waste number U211; Refrigerant R10; *Tetrachloorkoolstof* [Dutch]; *Tetrachloormetaan* [Dutch]; *Tetrachlorkohlenstoff, tetra* [German]; *Tetrachlormethan* [German]; Tetrachlorocarbon; Tetrachloromethane; Tetrachloromethane; *Tetrachlorure de carbone* [French]; *Tetrachlorure de carbone* [ISO-French]; *Tetraclorometano* [Italian]; *Tetracloruro di carbonio* [Italian]; Tetrafinol; Tetraform; Tetrasol; UN1846; Univerm; Vermoestricid

Materials used [CAS number]	Common and trade names
Cellulose nitrate [9004-70-0]	Ambroid; Amer-Glo (Celanese Plastics Corp, USA); Amerith (Celanese Plastics Corp, USA); Bexoid (Bx Plastics Ltd, UK); BK2-W; BK2-Z; Book Tex (Atlas Powder Co, USA); C 2018; CA 80-15; Campholoid (Japanese Cellulose Nitrate); Cascaphene (Cascelloid Ltd, UK); Cascelloid (Cascelloid Ltd, UK); Celastics (Celastic Corp, USA); Celex; Celloidin; Cellulac (British Plastoids Co, UK); Celluloid (Celanese Plastics Corp, USA); Cellulose Acetate Powder Made In France; Cellulose nitrate; Cellulose tetranitrate; Cellulose trinitrate; Cellulose, nitrate; Celluvarno (Sillcocks-Miller Co, USA); CN 85; Collodion; Collodion cotton; Collodion wool; Collodion, flexible; Colloxylin; Colloxylin VNV; Corial EM finish F; Daicel RS 1; Dai-Nippon Film (Japanese Motion Picture Film); Dentagiene (Canadian Industries Ltd,Canada); Duco Cement (Decon); Dumold (E.I.Du Pont De Nemours, USA); Durofix (Rawlplug); E 1440; Ercolene; Exonite (Dover Ltd, UK); Fiberlac (Monsanto Chemical Co, USA); Fiberloid (The Fiberloid Corp, USA); Fiberlon (The Fiberloid Corp, USA); Filac (Alfred Harris & Co, UK); Flexible collodion; Flexseal (Flexrock Co, USA); FM-Nts; Frigilene; Fulmicoton; Gemlike (Gemloid Corp, USA); Guncotton; H 1/2; Halex (Halex Ltd, UK); Hercules Cellulose; Herculoid (Hercules Powder Co, USA); HMG; HMG Heat and Waterproof Adhesive (Guest); HSDB 1973; HX 3/5; Hycoloid (Celluplastic Corp And Hygienic Tube & Container Co, USA); Invaleur (Celanese Plastics Corp, USA); Ivoride (Daniel Spill Co, UK); Keratol (Atlas Powder Co, USA); Kodafilm (Eastman Kodak Co, USA); Kodak LR 115; Kodaloid (Eastman Kodak Co, USA); LR 115; Lusteroid (Lusteroid Container Co, USA); Mural Rexine (Ici Ltd, UK); Necoloidin; Nitrate Flake (Hercules Powder Co, USA); Nitrocel S; Nitrocellulose; NITROCELLULOSE (CELLULOSE NITRATE); Nitrocellulose E950; Nitrocellulose solution; Nitrocellulose with alcohol not <25% alcohol, by mass, and not >12.6% nitrogen, by dry mass [UN2556] [Flammable solid]; Nitrocellulose with water not <25% water, by mass [UN2555] [Flammable solid]; Nitrocellulose, dry or wetted with < 25% water (or alcohol), by mass; Nitrocellulose, dry or wetted with <25% water (or alcohol), by mass [UN0340] [Explosive 1.1D]; Nitrocellulose, plasticized with not <18% plasticizing substance, by mass [UN0343] [Explosive 1.3C]; Nitrocellulose, solution, flammable with not >12.6% nitrogen, by mass, and not >55% nitrocellulose [UN2059] [Flammable liquid]; Nitrocellulose, unmodified or plasticized with < 18% plasticizing substance, by mass; Nitrocellulose, unmodified or plasticized with <18% plasticizing substance, by mass [UN0341] [Explosive 1.1D]; Nitrocellulose, wetted with not <25% alcohol, by mass [UN0342] [Explosive 1.3C]; Nitrocellulose, with not > 12.6% nitrogen, by dry mass, or Nitrocellulose mixture with pigment or Nitrocellulose mixture with plasticizer or Nitrocellulose mixture with pigment and [sic.]; Nitrocotton; Nitron; Nitron (Monsanto Chemical Co, USA & UK); Nitron (nitrocellulose); Nixon N/C; Nixonoid (Nixon Nitration Works, USA); NP 11; NTs 218; NTs 222; NTs 539; NTs 542; NTs 62; Oralite (Oralite Co, UK); Parlodion; Parlodion strips; Pentex (UK Plastics Ltd, UK); Permanite (Parker Pen Co, USA); Phoenixite (Japanese Celluloid); Pirossilina [DCIT]; Piroxilina [INN-Spanish]; Plastine (Sillcocks-Miller Co, USA. Also Black); Protecto (Celluloid Corp, USA); Proxyl (Lee S.Smith & Son Manufacturing, USA); Pyralin; Pyralin (E.I.Du Pont De Nemours, USA, but this trade name now refers to their polyimide resin); Pyra-Shell (Shoeform Co, USA); Pyroxilene; Pyroxylin; Pyroxylin [USAN:INN:JAN]; Pyroxylin solution; *Pyroxyline* [INN-French]; *Pyroxylinum* [INN-Latin]; R.S.Nitrocellulose; Radite (Shaeffer Pen Co, USA); Rexine (Ici (Rexine) Ltd, UK); RF 10; RS; RS 1/2; RS Nitrocellulose; Samson (Carpenter Steel Co, USA); Shadolac MT; Simco (Sillcocks-Miller Co, USA); Soluble gun cotton; Synpor; Tsapolak 964; UN0340; UN0341; UN0342; UN0343; UN2059; UN2555; UN2556; UN2557; Viscoloid (E.I.Du Pont De Nemours, USA); Xyloidin; Xylonite (David Spill Co, Later British Xylonite UK); Zaflex (Atlas Powder Co, USA); Zakaf (Atlas Powder Co, USA); Zapon Leathercloth (The Locomotive Rubber & Waterproofing Co, UK)
Chloroform [67-66-3]	[erroneously called "formyl trichloride"]; 1,1,1-Trichloromethane; 4-01-00-00042 (Beilstein Handbook Reference); AI3-24207; BRN 1731042; Caswell No. 192; CCRIS 137; Chloroform; Chloroform [UN1888] [Poison]; *Chloroforme* [French]; *Cloroformio* [Italian]; EINECS 200-663-8; EPA Pesticide Chemical Code 020701; Formyl trichloride; Freon 20; HSDB 56; Methane trichloride; Methane, trichloro-; Methenyl chloride; Methenyltrichloride; Methenyl trichloride; Methyl trichloride; NCI-C02686; NSC 77361; R 20; R 20 (Refrigerant); RCRA waste no. U044; RCRA waste number U044; Refrigerant R20; *Trichloormethaan* [Dutch]; *Trichlormethan* [Czech]; Trichloroform; Trichloromethane; *Triclorometano* [Italian]; UN1888
Enzyme active detergent	Alconox; Ariel (Procter and Gamble)l; Bio-ad; Biz; Bold 2-in-1 Aqua (Procter and Gamble); Borax; Calgon; Cheer; Daz (Procter and Gamble); dishwasher powder; enzyme-active laundry detergent; Ivory; Joy; Oxydol; Persil; Persil Performance (Lever); Sunfresh Surf (Lever); Tide; Vanish (Reckitt Benckiser)

Materials used [CAS number]	Common and trade names
Ethanol [64-17-5]	1-Hydroxyethane; Absolute ethanol; *Aethanol* [German]; *Aethylalkohol* [German]; AI3-01706; Alcohol; Alcohol (ethyl alcohol); Alcohol dehydrated; Alcohol, anhydrous; Alcohol, diluted; Alcohol, ethyl; Alcohols; *Alcool ethylique* [French]; *Alcool etilico* [Italian]; Algrain; *Alkohol* [German]; *Alkoholu etylowego* [Polish]; Anhydrol; Caswell No. 430; CCRIS 945; Cologne spirit; cologne spirits (alcohol); Denatured alcohol; Denatured alcohol CD-10; Denatured alcohol CD-5; Denatured alcohol CD-5a; Denatured alcohol SD-1; Denatured alcohol SD-13a; Denatured alcohol SD-17; Denatured alcohol SD-23a; Denatured alcohol SD-28; Denatured alcohol SD-30; Denatured alcohol SD-39b; Denatured alcohol SD-39c; Denatured alcohol SD-3a; Denatured alcohol SD-40m; Denatured ethanol; Distilled spirits; EINECS 200-578-6; EPA Pesticide Chemical Code 001501; *Etanolo* [Italian]; Ethanol; Ethanol 200 proof; Ethanol absolute; Ethanol solution; Ethanol, undenatured; Ethyl alcohol; Ethyl alcohol & water, 10%; Ethyl alcohol & water, 20%; Ethyl alcohol & water, 30%; Ethyl alcohol & water, 40%; Ethyl alcohol & water, 5%; Ethyl alcohol & water, 50%; Ethyl alcohol & water, 60%; Ethyl alcohol & water, 70%; Ethyl alcohol & water, 80%; Ethyl alcohol & water, 95%; Ethyl alcohol & water, 96%; Ethyl alcohol and water; Ethyl alcohol anhydrous; Ethyl alcohol in alcoholic beverages; Ethyl alcohol usp; Ethyl alcohol, undenatured; Ethyl hydrate; Ethyl hydroxide; *Ethylalcohol* [Dutch]; EtOH; *Etylowy alkohol* [Polish]; FEMA No. 2419; FEMA Number 2419; Fermentation alcohol; Grain alcohol; Grain alcohol; HSDB 82; Hydroxyethane; jaysol; Jaysol S; Methylcarbinol; Molasses alcohol; NCI-C03134; NSC 85228; Potato alcohol; Ru-Tuss Expectorant; Ru-Tuss Hydrocodone Liquid; Ru-Tuss Liquid; SD Alchol 23-hydrogen; sd alcohol 23-hydrogen; SDM No. 37; Spirit; Spirits of wine; Synasol; Tecsol; Tecsol C
Ether [60-29-7]	1,1'-Oxybisethane; 3-Oxapentane; Aether; AI3-24233; Anaesthetic ether; Anesthesia ether; Anesthetic ether; *Diaethylaether* [German]; Diethyl ether; Diethyl ether (Ethyl ether); Diethyl ether [Anaesthetics, volatile]; Diethyl ether or ethyl ether [UN1155] [Flammable liquid]; Diethyl oxide; *Dwuetylowy eter* [Polish]; EINECS 200-467-2; *Etere etilico* [Italian]; Ethane, 1,1'-oxybis-; Ether; Ether [JAN]; *Ether ethylique* [French]; Ether, ethyl; Ethoxyethane; Ethyl ether; Ethyl ether (8CI); Ethyl ether, tech.; Ethyl oxide; Ethylic ether; HSDB 70; NSC 100036; *Oxyde d'ethyle* [French]; Pronarcol; RCRA waste no. U117; RCRA waste number U117; Solvent ether; UN1155
Ethyl acetate [141-78-6]	*Acetate d'ethyle* [French]; *Acetato de etilo* [Spanish]; Acetic acid ethyl ester; Acetic acid, ethyl ester; Acetic ester; Acetic ether; Acetidin; Acetoxyethane; *Aethylacetat* [German]; AI3-00404; Caswell No. 429; CCRIS 6036; EINECS 205-500-4; EPA Pesticide Chemical Code 044003; *Essigester* [German]; Ethyl acetate; Ethyl acetate; Ethyl acetate (natural); Ethyl acetate [UN1173] [Flammable liquid]; Ethyl acetic ester; Ethyl ester; Ethyl ester of acetic acid; Ethyl ethanoate; Ethyl ethanoate; *Ethylacetaat* [Dutch]; Ethylacetate; *Ethyle (acetate d')* [French]; *Ethylester kyseliny octove* [Czech]; *Etile (acetato di)* [Italian]; FEMA No. 2414; HSDB 83; NSC 70930; *Octan etylu* [Polish]; RCRA waste no. U112; RCRA waste number U112; UN1173; Vinegar naphtha
Ethylene diaminetetracetic acid, disodium salt (EDTA) [139-33-3 (anhydrous); 6381-92-6 (dihydrate)]	(Ethylenedinitrilo)-tetraacetic acid disodium salt; Acetic acid, (ethylenedinitrilo)tetra-, disodium salt; AI3-18049; CBC 50152966; CCRIS 3658; Cheladrate; Chelaplex III; Chelaton 3; Chelaton III; Chelest 200; Chelest B; Clewat N; Complexon III; *Dinatrium ethylendiamintetraacetat* [Czech]; Disodium (ethylenedinitrilo)tetraacetate; Disodium (ethylenedinitrilo)tetraacetic acid; Disodium diacid ethylenediaminetetraacetate; Disodium dihydrogen ethylenediaminetetraacetate; Disodium dihydrogen(ethylenedinitrilo)tetraacetate; Disodium edathamil; Disodium edetate; Disodium EDTA; Disodium edta, anhydrous; Disodium ethylenediamine-N,N,N',N'-tetraacetate; Disodium ethylenediaminetetraacetate; Disodium ethylenediaminetetraacetic acid; Disodium N,N'-1,2-ethanediylbis(N-(carboxymethyl)glycine); Disodium salt of EDTA; Disodium sequestrene; Disodium tetracemate; Disodium versenate; Disodium versene; Diso-Tate; Dotite 2NA; DR-16133; *E.D.T.A. disodique* [French]; Edathamil disodium; Edetate disodium; Edetate sodium [USAN]; Edetic acid disodium salt; EDTA disodium; EDTA disodium salt; EINECS 205-358-3; Endrate disodium; Ethylene diamine tetraacetic acid, disodium salt; Ethylenebis(iminodiacetic acid) disodium salt; Ethylenediaminetetraacetate, disodium salt; Ethylenediaminetetraacetic acid, disodium salt; F 1; F 1 (complexon); F 1 (VAN); Glycine, N,N'-1,2-ethanediylbis(N-(carboxymethyl)-, disodium salt; Kiresuto B; Komplexon III; Mavacid ED 4; Metaquest B; N,N'-1,2-Ethanediylbis(N-(carboxymethyl)glycine) disodium salt; NSC 2760; Perma kleer 50 crystals disodium salt; Perma kleer di crystals; Selekton B 2; Sequestrene sodium 2; Sodium ethylenediaminetetraacetate; Sodium versenate; Tetracemate disodium; Titriplex III; Trilon BD; Triplex III; Veresene disodium salt; Versene NA; Versene Na2; Versonol 120; Zonon D
Gasoline [8006-61-9]	Antiknock gasoline; *Benzin* (German); Casing head gasoline; Cracked gasoline; EINECS 232-349-1; Gasolene; Gasoline; Gasoline (casinghead); Gasoline [UN1203] [Flammable liquid]; Gasoline, natural; High-octane gasoline; HSDB 6477; Light gasoline; Motor fuel; Motor Spirits; Natural gasoline; Natural gasoline (natural gas); Petrol; Petrol, natural; Petroleum distillates; Petroleum ether; Polymer gasoline; Pyrolysis gasoline; Reformed gasoline; Straight-run gasoline; third fraction obtained on distillation of petroleum or crude oil; UN 1203; UN1203; Unleaded gasoline (wholly vaporized); White gasoline
Gum arabic [9000-01-5]	Acacia; Acacia [JAN]; Acacia arabica gum africa; Acacia dealbata gum; Acacia gum; Acacia senegal; Acacia senegal l. willd gum west africa; Acacia solution; Acacia syrup; Arabic gum; Australian gum; CCRIS 322; EINECS 232-519-5; FEMA No. 2001; Gum acacia; Gum arabic; Gum arabic (Acacia senegal (L.) Willd.); Gum arabicum; Gum Dragon; Gum ovaline; Gum senegal; HSDB 1914; Indian gum; NCI-C50748; Senegal gum; Starsol No. 1; Wattle gum

Materials used [CAS number]	Common and trade names
Hydrogen peroxide [7722-84-1]	Albone; Albone 35; Albone 35CG; Albone 50; Albone 50°CG; Albone 70; Albone 70°CG; Albone DS; Auricome; Caswell No. 486AAA; CCRIS 1060; Dihydrogen dioxide; EINECS 231-765-0; Elawox; EPA Pesticide Chemical Code 000595; H2O2; High-strength hydrogen peroxide; Hioxy; Hioxyl; HSDB 547; Hydrogen dioxide; Hydrogen dioxide solution; Hydrogen peroxide; Hydrogen peroxide (> 52% conc.); Hydrogen peroxide (aqueous); Hydrogen peroxide (conc > 52%); Hydrogen peroxide (conc >52%); Hydrogen peroxide (H2O2); Hydrogen peroxide solution (DOT); Hydrogen peroxide solutions; Hydrogen peroxide solutions (over 60% but not over 70%); Hydrogen peroxide solutions (over 8% but not over 60%); Hydrogen peroxide, 20% to 60%; Hydrogen peroxide, 3%; Hydrogen peroxide, 30%; Hydrogen peroxide, 8% to 20%; Hydrogen peroxide, 90%; Hydrogen peroxide, aqueous solutions with >40% but not >60% hydrogen peroxide (stabilized as necessary) [UN2014] [Oxidizer]; Hydrogen peroxide, aqueous solutions with not <20% but not >40% hydrogen peroxide (stabilized as necessary) [UN2014] [Oxidizer]; Hydrogen peroxide, aqueous solutions with not <8% but <20% hydrogen peroxide (stabilized as necessary) [UN2984] [Oxidizer]; Hydrogen peroxide, solution; Hydrogen peroxide, solution, 3%; Hydrogen peroxide, solution, 30%; Hydrogen peroxide, solution, 35%; Hydrogen peroxide, stabilized or hydrogen peroxide aqueous solutions, stabilized with >60% hydrogen peroxide [UN2015] [Oxidizer]; Hydroperoxide; Inhibine; Interox; Kastone; Lensept; NSC 19892; Oxydol; Perhydrol; Perone; Perone 30; Perone 35; Perone 50; *Perossido di idrogeno* [Italian]; Peroxaan; Peroxan; Peroxide; Peroxide of hydrogen; *Peroxyde d'hydrogene* [French]; Puresept; Superoxol; T-Stuff; UN 2014 (20%-52%); UN 2015 (>52%); UN 2984 (8%-20%); UN2014; UN2015; UN2984; *Wasserstoffperoxid* [German]; *Waterstofperoxyde* [Dutch]
Industrial methylated spirit (IMS) [Ethanol 64-17-5 as above with the addition of Methanol 67-56-1]	AI3-00409; Alcohol, methyl; *Alcool methylique* [French]; *Alcool metilico* [Italian]; Bieleski's solution; Carbinol; Caswell No. 552; CCRIS 2301; Coat-B1400; Colonial Spirit; Colonial spirits; Columbian Spirit; Columbian spirits; EINECS 200-659-6; EPA Pesticide Chemical Code 053801; Eureka Products Criosine Disinfectant; Eureka Products, Criosine; Freers Elm Arrester; HSDB 93; Ideal Concentrated Wood Preservative; *Metanol* [Spanish]; *Metanolo* [Italian]; Methanol; Methanol, or methyl alcohol [UN1230] [Flammable liquid, Poison]; Methyl alcohol; Methyl alcohol (Methanol); Methyl hydrate; Methyl hydroxide; *Methylalkohol* [German]; Methylol; *Metylowy alkohol* [Polish]; Monohydroxymethane; NSC 85232; Pyroligneous spirit; Pyroxylic Spirit; Pyroxylic spirits; RCRA waste no. U154; RCRA waste number U154; Surflo-B17; UN1230; Wilbur-Ellis Smut-Guard; Wood; Wood alcohol; Wood naphtha; Wood Spirit; X-Cide 402 Industrial Bactericide
Kerosene [8008-20-6]	AF 100 (pesticide); Astral Oil; Avtur; Avtur (pesticide); Bayol 35; Bitumen Cutter; Caswell No. 517; CCRIS 1359; Coal oil; Deodorized base oil; Deodorized kerosene; Distillate fuel oils, light; EINECS 232-366-4; EPA Pesticide Chemical Code 063501; Escaid 100; Escaid 110; Exxsol D 200/240; Fuel No. 1 [Oil, fuel]; Fuel oil No. 1; fuel oil, no.5; Fuels, kerosine; HSDB 632; Ink oil; Jet A fuel; Jet Fuel JP-1; Jet fuels, JP-5; JP-5; JP5 Jet fuel; JP-5 Navy Fuel; Jp-5 navy fuel/marine diesel fuel; Kerosene; Kerosene (deodorized); Kerosene [UN1223] [Flammable liquid]; Kerosene, straight run; Kerosine; Kerosine (petroleum); Kerosine Burner Fuel; Kerosine, (petroleum); Kerosine, petroleum; KO 30 (solvent); Marine Diesel Fuel and JP-5 Navy Fuel; Mineral Colza; Mineral Seal; Navy Fuel JP-5; Navy fuels JP-5; Neochiozol; Nysolvin 75A; Odorless Solvent 3440; Paraffin (U.K.); Parasol; Pegasol 3040; Petroleum base oil; petroleum fuel; Range Oil; Range oil [Note: A refined petroleum solvent (predominantly C9-C16), which typically is 25% normal paraffins, 11% branched paraffins, 30% monocycloparaffins, 12% dicycloparaffins, 1% tricycloparaffins, 16% mononuclear aromatics & 5% dinuclear aromatics]; Range Oil JP-2; Range-oil; residual oil no.5; Shell 140; Shellsol 2046; Straight-run kerosene; SX 12; SX 7; UN1223
Linseed oil [8001-26-1]	*Aceite de Linaza*; Acid refined linseed oil; Acidulated linseed soapstock; Bodied linseed oil; Caswell No. 527A; EINECS 232-278-6; EPA Pesticide Chemical Code 031603; Fats and Glyceridic oils, flaxseed; Fats and Glyceridic oils, linseed; Flaxseed oil; Groco; HSDB 5155; Huile de Lin; L-310; Leinol; Linseed absolute; Linseed fatty acids, glycerin ester; Linseed oil; Linseed oil [Oil, misc.]; Linseed oil absolute; Linseed oil extract; Linseed oil fatty acids, glycerol triester; Linseed oil, alkali refined; Linseed oil, bleached; Linseed oil, wash recovered; Oil of Linseed; Oils, glyceridic, flaxseed or linseed; Oils, linseed; Oleum Lini; Sunflower oil
Mercury (II) chloride [7487-94-7]	Abavit B; Bichloride of mercury; *Bichlorure de mercure* [French]; Calochlor; Calo-Clor; Calocure; Caswell No. 544; CCRIS 4838; *Chlorid rtutnaty* [Czech]; *Chlorure mercurique* [French]; *Chlorure mercurique* [ISO-French]; *Cloruro di mercurio* [Italian]; Corrosive mercury chloride; Corrosive sublimate; Dichloromercury; EINECS 231-299-8; EPA Pesticide Chemical Code 052001; Fungchex; HSDB 33; Hydraargyrum bichloratum; Mercuric bichloride; Mercuric chloride; Mercuric chloride [ISO]; Mercuric chloride [JAN]; Mercuric chloride [Mercury and mercury compounds]; Mercuric chloride [UN1624] [Poison]; Mercuric chloride; Mercury (II) Chloride; Mercury bichloride; Mercury chloride; Mercury chloride (2); Mercury chloride ($HgCl_2$); Mercury dichloride; Mercury perchloride; Mercury(2+) chloride; Mercury(II) chloride; NCI-C60173; NSC 353255; Perchloride of mercury; *Quecksilber chlorid* [German]; *Sublimat* [Czech]; Sublimate; Sulem; *Sulema* [Russian]; TL 898; UN1624

Materials used [CAS number]	Common and trade names
Methylmethacrylate/ethylacrylate resin [80-62-6]	2-(Methoxycarbonyl)-1-propene; 2-Methyl-2-propenoic acid methyl ester; 2-methylacrylic acid methyl ester; 2-Methylacrylic acid, methyl ester; 2-Propenoic acid, 2-methyl-, methyl ester; 4-02-00-01519 (Beilstein Handbook Reference); Acryester M; Acrylic acid, 2-methyl-, methyl ester; Acrylic resin monomer; AI3-24946; BRN 0605459; CCRIS 1364; Diakon; EINECS 201-297-1; Elvacite 2044; HSDB 195; *Metakrylan metylu* [Polish]; *Methacrylate de methyle* [French]; Methacrylic acid methyl ester; Methacrylic acid, methyl ester; *Methacrylsaeuremethyl ester* [German]; Methyl 2-methyl-2-propenoate; Methyl 2-methylpropenoate; Methyl alpha-methylacrylate; Methyl ester of methacrylic acid; Methyl methacrylate; Methyl methacrylate monomer; Methyl methacrylate monomer, inhibited [UN1247] [Flammable liquid]; Methyl methacrylate; Methyl methylacrylate; *Methylester kyseliny methakrylove* [Czech]; *Methylmethacrylaat* [Dutch]; *Methyl-methacrylat* [German]; *Metil metacrilato* [Italian]; MMA; MME; Monocite methacrylate monomer; NCI-C50680; NSC 4769; Pegalan; RCRA waste no. U162; RCRA waste number U162; TEB 3K; UN1247
Oxalic acid [144-62-7 (anhydrous); 6153-56-6 (dihydrate)]	4-02-00-01819 (Beilstein Handbook Reference); *Acide oxalique* [French]; *Acido ossalico* [Italian]; Acidum oxalicum; AI3-26463; Aktisal; Aquisal; BRN 0385686; Caswell No. 625; CCRIS 1454; Dicarboxylic acid; EINECS 205-634-3; EPA Pesticide Chemical Code 009601; Ethane-1,2-dioic acid; Ethanedioic acid; Ethanedionic acid; HSDB 1100; *Kyselina stavelova* [Czech]; NCI-C55209; NSC 62774; *Oxaalzuur* [Dutch]; Oxalate; Oxalic acid; Oxalic acid (aqueous); Oxalic acid dihydrate; Oxalic acid solution, 10% W/V; *Oxalsaeure* [German]; Oxiric acid
Pepsin [9001-75-6]	A mixture of pepsin obtained from the gastric mucosa of hogs or cattle and lactose. It is an enzyme drug having a proteolytic activity; Allen &Hanburys' Liq. Pancreaticus; E.C. 3.4.1; E.C. 3.4.23.1; EINECS 232-629-3; Elixer lactate of pepsin; Gastric juice enzyme; Lactated pepsin; Lactated pepsin elixir; Pepsase; Pepsin; Pepsin A; Pepsin fortior; Pepsin NF; Pepsin, bovine; Pepsin, powder; Pepsinum; Saccharated pepsin [JAN]
Poly(vinyl) acetate (PVAC) [9003-20-7]	76 Res; Acetic acid ethenyl ester, homopolymer; Acetic acid vinyl ester, polymers; Acetic acid, ethenyl ester, homopolymer; Acetic acid, vinyl ester, polymer; Asahisol 1527; ASB 516; AYAA; AYAF; AYJV; Bakelite AYAA; Bakelite AYAF; Bakelite AYAT; Bakelite LP 90; Bond CH 1200; Bond CH 18; Bond CH 3; Booksaver; Borden 2123; Cascorez; Cemedine 196; Cevian 380; Cevian A 678; D 50; D 50 (Polymer); D 50 M; Danfirm; Daratak; DCA 70; Duvilax; Duvilax BD 20; Duvilax HN; Duvilax LM 52; Elmer's Glue All; Elvacet 81-900; Emultex F; En-cor; EP 1208; EP 1436; EP 1437; EP 1463; Esnil P 18; Ethenyl acetate homopolymer; Ethenyl acetate, homopolymer; Everflex B; Flexiplast (Foster - Grant Co, USA); Formvar 1285; Gelva; Gelva (Shawinigan Chemicals, UK); Gelva 25; Gelva CSV 16; Gelva GP 702; Gelva S 55H; Gelva TS 22; Gelva TS 23; Gelva TS 30; Gelva TS 85; Gelva V 100; Gelva V 15; Gelva V 25; Gelva V 800; Gohensil E 50Y; Gohsenyl E 50 Y; HSDB 1250; Kurare OM 100; Lemac; Lemac 1000; Lustrex (Foster - Grant Co, USA); Meikatex 5000NG60; Merckogel OR; Merckogen 6000; Mokotex D 2602; Movinyl; Movinyl 114; Movinyl 50M; Movinyl 801; Mowilith; Mowilith 30; Mowilith 50; Mowilith 70; Mowilith 90; Mowilith D; Mowilith DV; Mowilith M70; National 120-1207; National starch 1014; NS 2842; OM 100; OR 1500; P-170; Pioloform F; Plyamul 40-155; Plyamul 40-350; Polisol S-3; Poly(vinyl acetate); Poly(vinyl acetate), sec. stand., typical M.W.194800, typical M.N.63600; Poly(vinylacetate); Polyco 117FR; Polyco 2116; Polyco 2134; Polyco 953; Polyfox P 20; Polyfox PO; Polysol 1000; Polysol 1000AX; Polysol 1200; Polysol PS 10; Polysol S 5; Polysol S 6; Polyvinyl acetate; Polyvinyl acetate resin; Protex (polymer); PS 3h; PVAE; R 10688; Raviflex 43; Resyn 25-1014; Resyn 25-1025; Rhodopas; Rhodopas 010; Rhodopas 5000SMR; Rhodopas 5425; Rhodopas A 10; Rhodopas AM 041; Rhodopas B; Rhodopas BB; Rhodopas HV 2; Rohdopasm; Rhodopas M; RV225-5B; Sakunol SN 08; Setamul N6525; S-Nyl-P 42; Soloid; Solvar (Shawingan Products Corp, USA); Soviol; SP 60; SP 60 (Ester); Toabond 2; Toabond 40H; Toabond 6; TS2; Ucar 130; Ucar 15; UK 131; V 501; VA 0112; Vinac; Vinac ASB 10; Vinac B 7; Vinac RP251; Vinacet D; Vinalite D 50N; Vinalite DS 41/11; Vinamul 9300; Vinapol A 16; Vinipaint 555; Vinnapas B; Vinnapas B 100; Vinnapas B 17; Vinnapas UW 50; Vinyl acetate homopolymer; Vinyl Acetate Latex; Vinyl Acetate Resin; Vinyl Acetate, Polymer
Poly(vinyl) acetate/poly(vinyl) alcohol emulsion (PVAC/PVAL)	PVAC/PVAL; Elmer's Glue-All
Poly(vinyl) butyral resin [63148-65-2]	Bakelite XYHL; Butacite (E.I.du Pont de Nemours, USA); Butvar; Butvar (Shawingan Products Corp, USA); Butvar 76; Butvar 79; Butvar B 72; Butvar B 73; Butvar B 76; Butvar B 79; Butvar B 90; Butvar B 98; Butvar B-79; Butvar B-98; Butvel; Butyral (polymer); Butyral resins; Denka 6000°C; Denka Butyral; Denka Butyral 2000-1; Denka Butyral 2000-2; Denka Butyral 2000L; Denka Butyral 3000-1; Denka Butyral 3000-2; Denka Butyral 3000-4; Denka Butyral 3000K; Denka Butyral 4000; Denka Butyral 4000-1; Denka Butyral 4000-2; Denka Butyral 5000; Denka Butyral 5000A; Denka Butyral 6000; Denka Butyral 6000AP; Denka Butyral 6000AS; Denka Butyral 6000°C; Denka Butyral 6000°CG; Denka Butyral 6000EP; Denka Butyral 6000G; Denka Butyral 600°C; Poly(2-propyl-m-dioxane-4,6-diylene); Poly(vinyl butyral), fine granular powder; Polyvinyl alcohol, reaction product with butyraldehyde; Polyvinyl butyral; Polyvinyl butyral resin; Polyvinyl butyral resins; *Polyvinylbutyral* [Czech]; Saflex (Monsanto Chemical Co, USA); Vinyl acetal polymers; Vinyl acetal polymers, butyrals
Potassium carbonate (potash) [584-08-7]	Carbonate of potash; Carbonic acid, dipotassium salt; Caswell No. 685; CCRIS 7320; Dipotassium carbonate; EINECS 209-529-3; EPA Pesticide Chemical Code 073504; HSDB 1262; Kalium carbonicum; *Kaliumcarbonat* [German]; K-Gran; Pearl ash; Potash; Potasii carbonas; Potassium carbonate; Potassium carbonate (2:1); Potassium carbonate (K2(CO3)); Potassium carbonate (K2CO3); Potassium carbonate, anhydrous; Sal tartar; Salt of tartar; Salt of wormwood

Materials used [CAS number]	Common and trade names
Shellac [9000-59-3]	Button shellac; Candy glaze; Confectioner's glaze; EINECS 232-549-9; Gum lac; Lac resin; Lacca; Orange shellac; Resins, lac or shellac; Schellack; Shellac; Shellac Gum; Shellac orange S-40; Shellac, purified; White shellac
Sodium bicarbonate [144-55-8]	Acid sodium carbonate; Acidosan; Baking soda; Baros; Bicarbonate of soda; Carbonic acid monosodium salt; carbonic acid sodium salt (1:1); Carbonic acid, monosodium salt; Caswell No. 747; CCRIS 3064; Col-evac; Colyte; EINECS 205-633-8; EPA Pesticide Chemical Code 073505; HSDB 697; Jusonin; Meylon; Monosodium carbonate; Monosodium hydrogen carbonate; Natrii hydrogencarbonas; Natrium bicarbonicum; Natrium hydrogencarbonicum; Natriumhydrogenkarbonat; Natron [erroneously]; Neut; NSC 134031; Sel de vichy; Soda (van); Soda Mint; Sodium acid carbonate; Sodium bicarbonate; Sodium bicarbonate (1:1); Sodium bicarbonate [USAN:JAN]; Sodium carbonate (Na(HCO3)); Sodium hydrocarbonate; Sodium hydrogen carbonate; Sodium hydrogencarbonate; Soludal
Sodium carbonate (soda, washing soda) [497-19-8]	[Occurs in nature as the hydrate, thermonatrite, and the decahydrate, natron or natrite]; Ash; Bisodium carbonate; Calcined soda; Carbonic acid disodium salt; Carbonic acid sodium salt; Carbonic acid sodium salt (1:2); Carbonic acid, disodium salt; Caswell N0 752; CCRIS 7319; Crystol carbonate; Disodium carbonate; Disodium carbonate (Na2CO3); Dynamar L 13890; EINECS 207-838-8; EPA Pesticide Chemical Code 073506; HSDB 5018; Light Ash; Natrium Carbonicum Calcinatum; Natrium Carbonicum Siccatum; Na-X; NSC 156204; Sal soda; Snowlite I; Soda; Soda Ash; Soda Ash Light 4P; Soda, calcined; Sodium carbonate; Sodium carbonate (2:1); Sodium carbonate (Na2(CO3)); Sodium carbonate, anhydrous; Sodium Carbonate, Anhydrous ASTM D458; Sodium Carbonate, Anhydrous GE Materials D4D5; Solvay soda; Suprapur 6395; Trona; V 20N; V Soda; Washing soda
Sodium chloride [7647-14-5]	[Saline = 0.6% solution of sodium chloride, physiological salt solution]; Adsorbanac; Arm-A-Vial; Ayr; Caswell No. 754; CCRIS 982; Colyte; Common salt; Dendritis; EINECS 231-598-3; EPA Pesticide Chemical Code 013905; Extra Fine 200 Salt; Extra Fine 325 Salt; Flexivial; Gingivyl; H.G. blending; Halite; HSDB 6368; Hypersal; Hyposaline; Iodized salt; NaCl; *Natriumchlorid* [German]; NSC 77364; Purex; Rock salt; Saline; Saline solution; Salt; Salt (ingredient); Sea salt; Slow Sodium; Sodium chloric; Sodium chloride; Sodium chloride (Na4Cl4); Sodium chloride (NaCl); Sodium chloride [USAN:JAN]; Sodium chloride brine, purified; Sodium chloride, 99.999%; Sodium monochloride; SS salt; Stat trak plus; Sterling; Table salt; Top flake; Trisodium trichloride; White crystal
Sodium hydroxide [1310-73-2]	Aetznatron; Ascarite; Augus Hot Rod; Caswell No. 773; Caustic soda; Caustic soda solution; Caustic soda, liquid; Collo-Grillrein; Collo-Tapetta; EINECS 215-185-5; EPA Pesticide Chemical Code 075603; Fuers Rohr; HSDB 229; *Hydroxyde de sodium* [French]; Lewis-red devil lye; Liquid-plumr; Lye; lye, caustic; Natrium causticum; *Natriumhydroxid* [German]; Natrium-hydroxid, reinstes; *Natriumhydroxyde* [Dutch]; NSC 135799; Plung; Rohrputz; Rohrreiniger Rofix; Soda lye; Soda, caustic; Soda, hydrate; Soda, kaustische; Sodii hydroxidum; *Sodio(idrossido di)* [Italian]; Sodium hydrate; Sodium hydrate solution; Sodium hydroxide; Sodium hydroxide (Na(OH)); Sodium hydroxide (Na2(OH)2); Sodium Hydroxide [USAN]; Sodium hydroxide dimer; Sodium hydroxide solution; Sodium hydroxide, solid [UN1823] [Corrosive]; Sodium hydroxide, solution [UN1824] [Corrosive]; *Sodium(hydroxyde de)* [French]; UN 1823 (solid); UN 1824 (solution); UN1823; UN1824; White caustic; White caustic solution
Sodium hypochlorite (bleach) [7681-52-9]	AD Gel; Antiformin; B-K; B-K liquid; bleach; Carrel-dakin solution; Caswell No. 776; CCRIS 708; Chlorinated water (sodium hypochlorite); Chloros; Chlorox; Cloralex; Cloropool; Clorox; Clorox liquid bleach; Dakins solution; Dakin's solution; Deosan; Deosan Green Label Steriliser; Dispatch; Eau de javelle; EINECS 231-668-3; EPA Pesticide Chemical Code 014703; Hospital Milton; Household bleach; HSDB 748; Hychlorite; Hyclorite; Hypochlorite sodium; Hypochlorous acid, sodium salt; Hyposan and Voxsan; Hypure; Hypure N; Javel water; Javelle water; Javex; Klorocin; Liquid bleach; Mera industries 2MOM3B; Milton; Milton Crystals; Modified dakin's solution; Neo-cleaner; Neoseptal CL; Parozone; Piochlor; Purin B; Showchlon; Sodium hypochlorite; Sodium hypochlorite (NaClO); Sodium hypochlorite (NaOCl); Sodium hypochlorite [Hypochloride salts]; Sodium hypochlorite [USAN:JAN]; Sodium hypochlorite solution; Sodium hypochlorite solution (15% or less); Sodium hypochlorite, 13% active chlorine; Sodium oxychloride; Solutions, Dakin's; Sunnysol 150; Surchlor; UN 1791; XY 12; Youxiaolin
Sodium perborate [7632-04-4]	Dexol; EINECS 231-556-4; HSDB 1676; Perboric acid (HBO(O2)), sodium salt; Perboric acid (HBO3), sodium salt; Perboric acid, sodium salt; Peroxydol; Sodium perborate; Sodium perborate (BaBO3); Sodium peroxoborate; Sodium peroxoborate, anhydrous [UN3247] [Oxidizer]; Sodium peroxometaborate; UN3247
Toluene [108-88-3]	AI3-02261; Antisal 1a; Benzene, methyl-; Caswell No. 859; CCRIS 2366; CP 25; EINECS 203-625-9; EPA Pesticide Chemical Code 080601; HSDB 131; Methacide; Methane, phenyl-; Methyl benzene; Methyl benzol; Methylbenzene; methyl-Benzene; Methylbenzol; Monomethyl benzene; NCI-C07272; NSC 406333; phenyl methane; Phenylmethane; RCRA waste no. U220; RCRA waste number U220; Tol; *Tolueen* [Dutch]; *Toluen* [Czech]; Toluene; Toluene [UN1294] [Flammable liquid]; Toluene; *Tolueno* [Spanish]; Toluol; Toluol; *Toluolo* [Italian]; Tolu-Sol; UN 1294; UN1294

Materials used [CAS number]	Common and trade names
Trichloroethylene [79-01-6]	1,1,2-trichloroethene; 1,1,2-Trichloroethylene; 1,1-dichloro-2-chloroethylene; 1,2,2-trichloroethylene; 1-chloro-2,2-dichloroethylene; 1-chloro-2-dichloroethylene; 4-01-00-00712 (Beilstein Handbook Reference); Acetylene trichloride; AI3-00052; Algylen; Anamenth; Benzinol; Blacosolv; Blancosolv; BRN 1736782; Caswell No. 876; CCRIS 603; Cecolene; Chlorilen; Chlorylea; Chlorylen; Chorylen; Circosolv; Crawhaspol; Densinfluat; Dow-tri; Dukeron; EINECS 201-167-4; EPA Pesticide Chemical Code 081202; Ethene, trichloro-; Ethinyl trichloride; Ethylene trichloride; Ethylene, trichloro-; F 1120; Fleck-flip; Flock flip; Fluate; Gemalgene; Germalgene; HSDB 133; Lanadin; Lethurin; Narcogen; Narkogen; Narkosoid; NCI-C04546; Nialk; NSC 389; Per-A-Clor; Perm-a-chlor; Perm-a-clor; Petzinol; Philex; R 1120; RCRA waste no. U228; RCRA waste number U228; TCE; TCE (chlorohydrocarbon); Threthylen; Threthylene; Trethylene; Tri; Triad; Trial; triasol; *Trichlooretheen* [Dutch]; *Trichloorethyleen, tri* [Dutch]; *Trichloraethen* [German]; *Trichloraethylen, tri* [German]; Trichloraethylenum; Trichloran; Trichlorathane; Trichloren; Trichlorethylene; *Trichlorethylene, tri* [French]; Trichlorethylenum; Trichloroethene; Trichloroethylene; Trichloroethylene (IUPAC); Trichloroethylene (TCE); Trichloroethylene [INN]; Trichloroethylene [UN1710] [Poison]; *Trichloroethylenum* [INN-Latin]; Triciene; Tri-Clene; *Tricloretene* [Italian]; Tricloroetilene [DCIT]; *Tricloroetilene* [Italian]; *Tricloroetileno* [INN-Spanish]; Trielene; Trielin; *Trielina* [Italian]; Trieline; Triklone; Triklone N; Trilen; Trilene; Trilene TE-141; Triline; Trimar; Triol; Tri-plus; Tri-plus m; UN 1710; UN1710; Vestrol; Vitran; Westrosol
Turpentine [8006-64-2]	Caswell No. 900; Dipanol; EINECS 232-350-7; EPA Pesticide Chemical Code 084501; FEMA No. 3089; Gum spirits of turpentine; Gum turpentine; Gumspirits; HSDB 204; Kautschin; Oil of turpentine; Oil of turpentine, distillation residue; Oil of turpentine, rectified; Pine-cone oil; pinene, all isomers; Spirit of turpentine; Spirits of turpentine; Steam distilled turpentine; Sulfate turpentine; Sulfate wood turpentine; Terebenthene; *Terebenthine* [French]; Terebinthina; Terpene; *Terpentin oel* [German]; Terpentine; Turpentine; Turpentine (wood); Turpentine [UN1299] [Flammable liquid]; Turpentine oil; Turpentine oil, rectified; Turpentine oil, rectifier; Turpentine spirits; Turpentine steam distilled; Turpentine substitute [UN1300] [Flammable liquid]; Turpentine, oil; Turpentine, steam-distilled (Pinus spp.); Turps; UN1299; UN1300; Wood turpentine
White spirit (naptha or Stoddard's solvent) [8052-41-3]	Caswell No. 802; Dry cleaning safety solvent; EINECS 232-489-3; EPA Pesticide Chemical Code 063504; High flash naphtha; HSDB 7171; Mineral spirits; Mineral Spirits, Type I; Naphtha, solvent; Naphtha, stoddard solvent; Organic solvents, Stoddard solvent; PDF; Petroleum Distillate Fractions; Petroleum distillates; Petroleum solvent; Safety solvent naphtha; Solvents, naphthas; Spotting naphtha; Stoddard Solvent; Turpentine substitute; Varsol 1 (Exxon); White spirit; White spirits
Xylene [1330-20-7]	4-05-00-00951 (Beilstein Handbook Reference); AI3-02209-X; Benzene, dimethyl-; BRN 1901563; Caswell No. 906; CCRIS 903; Dimethylbenzene; Dimethylbenzene (mixed isomers); Dimethylbenzenes; EINECS 215-535-7; EPA Pesticide Chemical Code 086802; except p-xylene, mixed or all isomers; HSDB 4500; *Ksylen* [Polish]; m & p-xylene; m-,p-,o-Xylene; Methyl toluene; Methyltoluene; NCI-C55232; o-,m-,p-Xylene; RCRA waste no. U239; RCRA waste number U239; Socal aquatic solvent 3501; Total xylenes; UN 1307; Violet 3; *Xiloli* [Italian]; Xylene; Xylene (mixed isomers); Xylene (mixed); Xylene (o-, m-, p-isomers); Xylene (o,m,p isomers); Xylene (o-,m-,p-); Xylene mixture; Xylene mixture (60% m-xylene, 9% o-xylene, 14% p-xylene, 17% ethylbenzene); Xylene mixture (m-xylene, o-xylene, p-xylene); Xylene, (total); Xylene, isomers; Xylene, mixed; Xylene, mixed isomers, pure; xylene, mixed or all isomers, except p-; *Xylenen* [Dutch]; Xylenes; Xylenes (isomers and mixture); Xylenes (mixed); Xylenes (o-, m-, p-isomers); Xylenes mixed isomers; Xylenes; Xylenes, total; Xylol; *Xylole* [German]

Table 4. Materials used in the screening experiment, with CAS numbers [in brackets], and a list of common and trade names associated with each compound (CambridgeSoft Corporation 2004; Clydesdale 1982; Grant 1969; National Institute for Occupational Safety and Health 2005; Selwitz 1988; U.S. National Library of Medicine 2004; Zauscinski 2006).

With a clearer understanding of the terminology used to identify chemicals historically, it was possible to assess chemical usage for different treatment purposes. Treatments were grouped into the following categories: acid preparation, adhesive, adhesive for spirit collections, barrier coat, bleaching agent, chelating agent, cleaning agent, consolidant, degreasing agent, dry soft tissue preservative, drying agent, finishing materials, fungicide, moulding/casting materials, packing material, pesticide, photographic aid, sealant, skeleton preparation, solvent, and wet soft tissue preservative. Definitions for each of these treatments can be found in the Glossary. The documented uses of each of the compounds listed in Table 4 are outlined in Table 5.

Use: / Chemical [CAS number]:	Acid preparation:	Adhesive for spirit collections:	Adhesive:	Barrier coat:	Bleaching agent:	Chelating agent:	Cleaning agent:	Consolidant:	Degreasing agent:	Dry soft tissue preservative:	Drying agent:	Finishing materials:	Fungicide:	Moulding/casting materials:	Packing material:	Pesticide:	Photographic aid:	Sealant:	Skeleton preparation:	Solvent:	Wet soft tissue preservative:
1:1 IMS [64-17-5]:acetone [67-64-1]																				X	
1:1 Ether [60-29-7]:ethanol [64-17-5]																				X	
Acetic acid [64-19-7]	X									X											X
Acetone [67-64-1]			X	X			X	X	X		X	X		X						X	X
Acrylic dispersion/emulsion			X					X													
Aluminium potassium sulfate (alum) [10043-67-1 (anhydrous); 7784-24-9 (dodecahydrate)]										X						X			X		X
Ammonium hydroxide [1336-21-6]					X		X		X	X						X	X		X		X
Amyl acetate [628-63-7]								X												X	
Arsenic (arsenic trioxide) [1327-53-3]										X		X				X			X		X
Benzene [71-43-2]			X				X	X	X		X					X			X	X	
Carbon tetrachloride [56-23-5]					X			X	X							X				X	
Cellulose nitrate [9004-70-0]		X	X	X				X				X									
Chloroform [67-66-3]			X	X					X							X				X	X
Enzyme active detergent					X		X			X									X		
Ethanol [64-17-5]								X		X			X			X				X	X
Ethyl acetate [141-78-6]			X	X				X								X				X	
Ethylene diaminetetracetic acid, disodium salt (EDTA) [139-33-3 (anhydrous); 6381-92-6 (dihydrate)]	X					X	X														
Gasoline [8006-61-9]							X	X	X	X						X				X	
Gum arabic [9000-01-5]			X					X		X											
Hydrogen peroxide [7722-84-1]					X	X	X		X										X		X
Industrial methylated spirit (IMS) [64-17-5]			X					X	X		X									X	X
Kerosene [8008-20-6]				X			X	X						X						X	
Linseed oil [8001-26-1]								X		X		X						X			
Mercury (II) chloride [7487-94-7]										X			X	X		X					X
Methylmethacrylate/ethylacrylate resin [80-62-6]			X	X				X				X									
Oxalic acid [144-62-7 (anhydrous); 6153-56-6 (dihydrate)]						X	X			X											
Pepsin [9001-75-6]																			X		
Poly(vinyl) acetate emulsion (PVAC) [9003-20-7]			X					X													
Poly(vinyl) acetate/poly(vinyl) alcohol emulsion (PVAC/PVAL)			X					X													
Poly(vinyl) butyral resin [63148-65-2]			X				X	X				X									
Potassium carbonate (potash) [584-08-7]							X			X						X			X		X
Shellac [9000-59-3]			X	X				X		X		X		X				X			
Sodium bicarbonate [144-55-8]					X				X										X		X
Sodium carbonate (soda, washing soda) [479-19-8]					X		X		X	X						X			X		X
Sodium chloride (salt) [7647-14-5]										X					X				X		X
Sodium hydroxide [1310-73-2]							X		X	X									X		X
Sodium hypochlorite (bleach) [7681-52-9]					X														X		
Sodium perborate [7632-04-4]									X										X		
Sodium sulfide [1313-82-2]							X		X										X		
Toluene [108-88-3]			X	X				X												X	
Trichloroethylene [79-01-6]									X							X			X		
Turpentine [8006-64-2]		X					X		X	X		X								X	
White spirit (naptha or Stoddard's solvent) [8052-41-3]				X			X	X	X							X				X	
Xylene [1330-20-7]			X					X	X			X					X			X	

Table 5. Chemicals tested and their historic uses.

In summary, preparation and conservation treatments have a complex history. A wide range of treatments have been applied to collection materials for different reasons over time. Specimens may have been re-processed or may have moved between different collections, and different treatment traditions exist within the various fields interested with human and animal hard and soft tissues. Furthermore, the evolution of treatments in different disciplines and differences in terminology as well as trade names and common names associated with preparation and conservation treatments can make understanding the treatment history of a specimen less than straightforward. Identifying the range of methods and materials used in caring for hard and soft tissue collections in the past was one of the initial objectives of this study in order to begin to assess the effects of some of the most commonly documented treatments on DNA.

Chapter 4. Materials and methods

Following a review of the existing publications regarding the effects of preparation and conservation treatments on DNA and an extensive literature review to identify preparation and conservation treatments used in the past on human and animal hard and soft tissues, a two-part experimental assessment of the effects of treatments on DNA was instigated. First, a screening test was designed to compare the effects on DNA *in vitro* on a wide range of the most commonly documented treatments identified in the literature review. Although a few previous studies addressed the effects of treatments on freshly collected specimens, the methods used to identify damage was best suited for well preserved high molecular weight DNA, which would be unlikely to survive in any great quantity in the majority of specimens from archaeological contexts or from specimens held in collections for a substantial period of time. Therefore, a method was devised to identify and quantify damage to short strands of DNA.

The second part of this study consisted of a case study using both ancient and recent Egyptian animal mummies to assess the effects of mummification on DNA, as mummification can be considered one of the first preparation and conservation treatments. There is much debate surrounding the viability of ancient Egyptian mummies for DNA analyses, and it was hoped that some insight into the role mummification may play in the preservation of DNA could be gained by this study.

4.1. Screening test

The objective of the screening test was to identify DNA damage in the form of strand breakage resulting from treatment with an individual chemical commonly used in preparation and conservation treatments. In designing the screening test, it was considered important to test as many chemicals as possible and to be able to identify and quantify damage induced by treatment on short strands of DNA. It was therefore essential to account for the starting concentration of known length DNA fragments, and to control as many variables as possible to ensure that any damage identified was solely due to an individual chemical species. Existing methods used in previous studies to assess the effects of treatments on DNA in either medical pathology archival tissues or freshly collected biological specimens were considered inadequate (see Chapter 2), so a new method was devised.

Rather than using archaeological material as samples, in which the starting concentration of particular sequences could not be known and would be subject to variation based on sample size, fluorescently-labelled PCR products of a known length similar to that expected of ancient DNA (approximately 100-200bp) were used. By using PCR products, the starting concentration of the samples tested could be standardised, and loss of the DNA target sequence due to strand

breakage measured using capillary electrophoresis (CE). PCR products of standardised concentration allowed for optimally comparable results across treatments. As PCR was not used as a part of the method following DNA exposure to chemical treatment, contamination was not considered a major problem for this research. Most importantly, the effects of preparation and conservation treatments on DNA can be directly measured, enabling the identification of treatments that are more or less damaging to DNA.

To measure the degree of DNA damage in each sample, two DNA stock solutions were made from the PCR products generated for this study. Standardised samples of what was called the "test stock" were set up in a 96-well plate. To each well was added a different chemical treatment stock solution, which was allowed to incubate for a set period of time, after which the second "standard stock" was added to samples. A protocol to remove the treatment chemicals and precipitate the DNA in a standardised volume for each sample was then followed. Samples were analysed by CE, where peak height measurements were used to assess changes to DNA concentration, and the ratio between the test stock and standard stock peak heights was calculated for each sample. This peak height ratio would represent the damage sustained to the test stock during treatment in the screening test (any damage resulting from post-treatment sample preparation would affect both stocks equally).

Finally, several methods of data analysis, both qualitative and quantitative, were used to ensure consistency in results reporting. Having observed that an existing predictive model based on existing nucleic acid chemistry knowledge (Brown 1999) was not consistent with experimental results (Kigawa et al. 2003; Williams 1999; see also section 2.3), no predictions were made regarding the expected effects of the treatments tested in this study. Therefore, two-tailed tests for significance were used in all statistical anlyses.

For a generalised overview of the approaches taken in this experiment, see Figure 2.

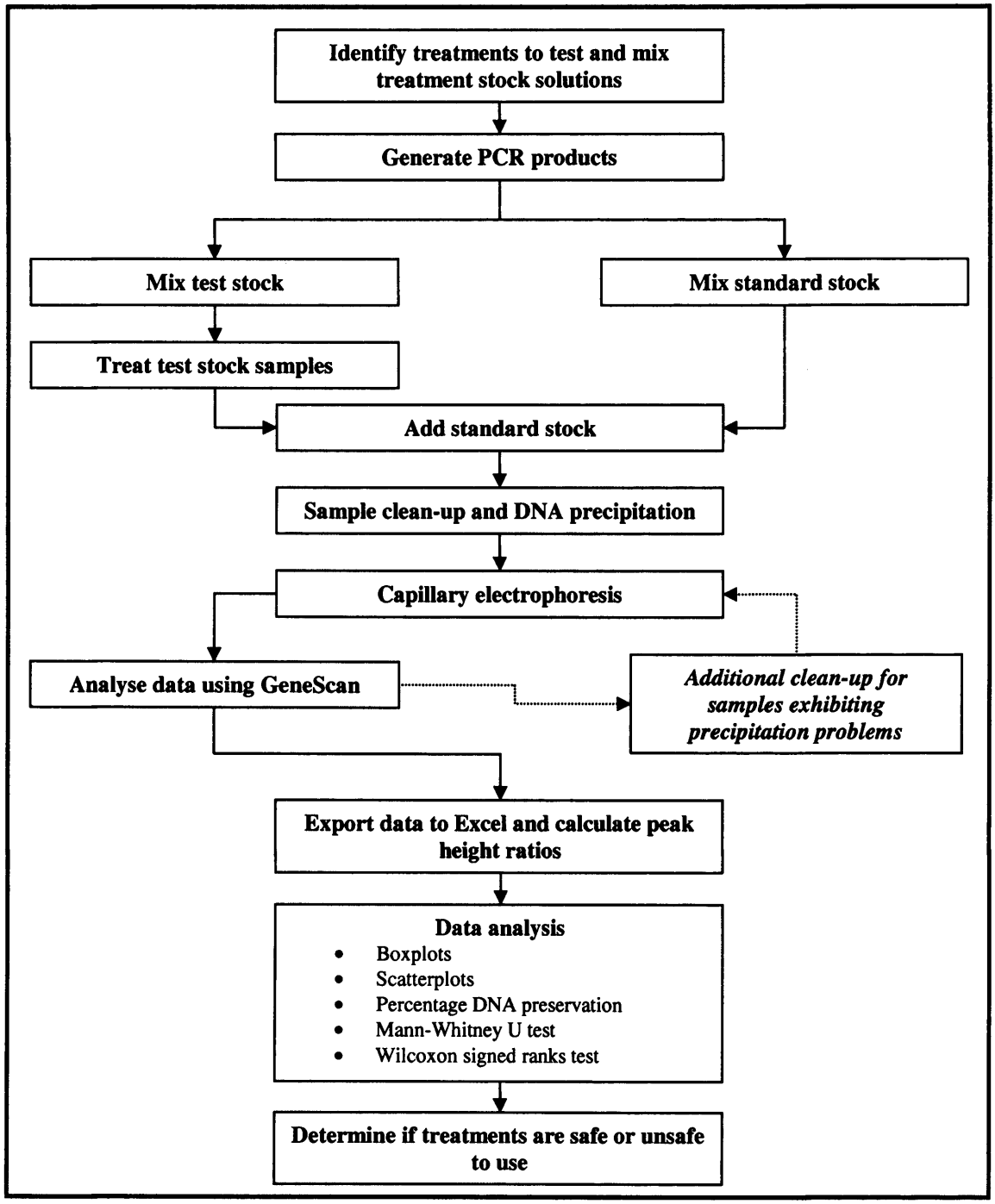


Figure 2. Generalised flowchart outlining major steps involved in the screening test.

4.1.1. Treatments tested

It was not possible to test all of the chemicals identified in the literature review that have been used on human and animal hard and soft tissues in the past. However, it was desirable to test a wide range of commonly used and well-documented treatments in order to get a broad idea of the potential preservation of DNA in existing collections. The number of treatments selected for testing was based on a practical consideration; the samples were placed in a 96-well plate for the duration of the experiment. Testing 96 different treatments was considered impractical, as 4 replicate samples of each treatment were to be tested to ensure consistency across samples, and processing 4 plates simultaneously was not ideal. It was decided to fit two full sets of

treatments onto a single plate including the necessary controls, therefore, 44 treatments could be tested, half of which were aqueous and half of which were organic solvent-based. The selection of 44 treatments to test out of the approximately 475 materials identified in the literature review was based on several criteria, which are summarised in Table 6.

Criteria for treatment selection:
• Compound/active ingredient was identifiable
• Compound/active ingredient was currently available and legal
• Treatment must be applied in liquid phase
• Clear instructions for use were provided, including
- Method of application (liquid)
- Amount or concentration applied
- Exposure time for treatment provided, when applicable
- Temperature for treatment provided, when applicable
• Referred to several times, with similar instructions found in more than one reference

Table 6. Summary of criteria used for selecting treatments to test.

The first criterion for selecting treatments to test was that the compound or active ingredient must be identifiable. Proprietary products mentioned by trade name only were often no longer manufactured, and the active ingredient could not be identified. Of the approximately 475 chemicals identified as having been used, roughly 40 were eliminated with this criterion.

The compound or active ingredient also had to be available and legally obtainable in the U.K. This ruled out several of the materials used for pest control in the past, such as dichloro-diphenyl-trichloroethane (DDT) and dichlorvos, amongst others.

The decision to use PCR products *in vitro* as the source DNA to be treated (see section 4.1.2) also influenced the selection of treatments tested. As small volumes of PCR products suspended in water were to be treated, treatments could not be carried out as they would traditionally be done on objects. Equal volumes of treatments had to be administered and treatments had to be applied by pipette. Therefore, only treatments administered in liquid form were selected in order to replicate the treatment conditions as closely as possible. This criterion excluded a number of treatment types, such as the application of dry powders or rubs often used in preparing hides and furs, as well as aerosols, vapours or gas fumigation.

Clear instructions for administering the treatments were also required, which included describing the method of application, any special directions for making up solutions, such as specifying the amount or concentration of the chemical, as well as the exposure time and any temperature requirements for the treatment, if applicable. If instructions were not provided enabling replication of the treatment with some degree of reliability, the treatment simply could

not be included in this study. Additionally, if a particular chemical was only found to be used in fixatives and/or preservative solutions but not any other treatment type, they were excluded from consideration because such materials have been and currently are under investigation by other researchers (Carter pers. comm. 2003, 2004), and such treatments are typically used on freshly collected material with the aim of preserving high molecular weight DNA, which is not the subject of this research. This criterion eliminated a large number of treatments.

For a chemical to be included in this study, it was also necessary to document several references to its use, with more than one reference to a specific treatment type recorded (including the chemical, method of treatment, concentration and exposure time) in order to suggest a trend of widespread use, rather than being an isolated case. Additionally, organic solvents are typically used as a carrier for a compound that was not water-soluble. When such treatments were tested, the organic solvent was tested separately to determine if any effects observed in the combined treatment could be due to the organic solvent alone. Organic solvents were sometimes used on their own as a treatment and were included for testing if referred to eight or more times in the literature either for use on their own or as a component of a treatment solution. Mineral oil was used in this experiment as an indicator of damage, as preliminary tests demonstrated that its use for 7 days was damaging to DNA, and its inclusion aided the identification of other treatments that were also damaging.

After compiling a shortlist of chemicals based on these criteria, one specific set of treatment conditions was selected for each chemical. When chemicals had been used at a variety of concentrations or for varying lengths of exposure, the highest "normal" concentration was selected. This was determined by excluding any outliers where a single reference advocated an extremely high concentration compared to the majority of references suggesting a more moderate concentration. When a treatment was cited by another author using different wording or even misquoting the original reference, this was considered a new treatment and the conditions considered, unless the misprint made repeating the treatment impossible, and the original author was also recorded to allow the evolution of specific treatments to be followed.

Aqueous treatment stocks were mixed using water purified by reverse osmosis from the Institute of Archaeology conservation laboratory. Treatment stocks were mixed at 50% higher concentration than the published concentration, as the water present in the DNA test stock would adjust the treatment concentration to the appropriate level. Further adjustments were required for particularly viscous treatment stock solutions, as Gilson pipettes could not accurately measure gum arabic and excess treatment was added to samples. Cellulose nitrate was further diluted to facilitate pipetting, and therefore was not administered at the concentration suggested in the literature. Some substitutions were also necessary, for example,

gasoline used for treatment in 1932 (Anderson) was invariably leaded (although this was unspecified), leaded gasoline is now difficult to obtain in the UK, so unleaded was used. In the case of enzymatic maceration, pepsin was used as a substitute for trypsin, as they are used under similar conditions (Hangay and Dingley 1985). Also, if a less hazardous version of a treatment was recorded, it was selected for testing, such as immersion in carbon tetrachloride at room temperature rather than heating a pot of carbon tetrachloride to 170°F in a double boiler on a gas stove (Rowley 1925: 213).

Few references provided specific treatment exposure times. If no individual reference for a particular chemical provided enough details for experimental replication, the relevant information from two similar references was combined. Treatments with a published exposure time of 12 hours were left overnight (approximately 18 hours). If a treatment was to be repeated, the sum total of the exposure time was used in the experiment, for example, Mooney et al. (1982: 125) recommended simmering formalin-fixed human skulls in enzyme active detergent for 8 hours per day for 3-5 days, so for the purposes of this experiment this treatment was carried out for 40 hours. If an organic solvent-based treatment was used for a longer period than the solvent was used alone for another purpose, the longest period the solvent would have been in contact with collection material was tested in this study. For comparability, treatment with acrylic dispersion and poly(vinyl) acetate (PVAC), and treatment with poly(vinyl) acetate/poly(vinyl) alcohol emulsion (PVAC/PVAL, specifically Elmer's glue) was overnight, as a specific exposure time for immersion was not given. For the experiment, if treatment instructions were vague or not provided, exposure times were as follows: "a few days" = 1 week, "a few hours" = overnight (18 hours), "simmer" = 6 hours/80°C (Mooney et al. 1982, 125), organic solvent bone immersion and all soft tissue surface treatments = 2 hours, bone surface treatments = 1 hour. Sodium perborate treatment was to be heated to boiling and allowed to cool overnight to room temperature, which was replicated by heating sample tubes to 100°C in a thermal cycler for 1 minute, and allowing it to cool to room temperature overnight. A maximum of one week treatment time was set. To keep to a viable laboratory schedule for mass processing of all samples, the end time of the experiment was set for 12.00 on the seventh day, with the addition of treatment solutions to samples timed so all experiments ended simultaneously.

Treatments were carried out at room temperature unless otherwise noted. Instructions for a few chemicals called for treatments to be carried out at an elevated temperature. Throughout this research, these treatments are referred to as "heated" treatments. To account separately for the effects of the chemical and of heating the treatment, unheated samples of the same treatments were also carried out, and were maintained under the same conditions as the other unheated treatments, and water control samples were also heated. All heated treatments were carried out

at either 37°C or 80°C, as only two ovens were available.

The chemicals (and CAS numbers) and treatment conditions selected for testing in this study, based on the criteria as set out above, are listed in Table 7. Both the experimental conditions and the published treatment conditions and instructions are listed, as well as the source of the chemical and any product numbers (Prod:), lot numbers (Lot:) or other relevant information (e.g. EC numbers, grade or purity of chemical, etc.). The material (e.g. bone, hide, etc) on which the specific treatment was used as well as the collection type the material was from is also listed as documented in the original reference, but application of the treatment may not have been restricted to these materials and collections in the past.

Chemical tested [CAS number]	Solvent	Material treated	Collection type	Published concentration (%)	Published exposure time	Published temperature	Instructions and Comments	Reference	Experimental concentration (%)	Experimental exposure time	Experimental temperature	Chemical source information
Acetic acid [64-19-7]	water	Fossil/Bone	Palaeontology/Zoology	15%			Acid preparation: ... acetic acid (about 15%). The specimen is allowed to soak in the acid bath until it is free of matrix. It is then placed in several changes of water and the specimen dried. ...The bone is then impregnated with polybutyl methacrylate and allowed to dry	(Satyamurti 1967: 16)	22.5% v/v	1 hour	Room temperature	BDH Laboratory Supplies, AnalaR, 100% Acetic Acid, Prod: 10001CU, Lot: K31738717 307, EC: 200-580-7
Acetone [67-64-1]		Bone	Zoology	100%	few hours		Degreasing agent: Suitable de-fatting agents are undiluted acetone, benzene, xylene or chloroform. A few hours immersion will suffice...	(Harris 1959: 223)	100%	overnight (18 hours)	Room temperature	BDH Laboratory Supply, AnalaR, 99.5%, Prod: 100034Q, Lot: K28042206, EC: 200-662-2
Acrylic dispersion/ emulsion	water	Bone	Archaeology	25%			Consolidant: for damp or wet material, use Acrysol WS-24 (Rohm and Haas), or equivalent 25%	(Storch 2003: 4)	37.5% v/v	overnight (18 hours)	Room temperature	Conservation Resources, Primal WS24 (Acrysol WS24)
Aluminium potassium sulfate (alum) [10043-67-1 (anhydrous); 7784-24-9 (dodecahydrate)]	water	Skin/fur	Zoology	9% w/v	3 days - 3 weeks or longer		Dry preservation of soft tissue: relaxing dry skins - immerse in weak but clean salt-and-alum bath for three days-three weeks, can initially soak in clean warm water briefly; can leave in salt and alum bath as long as desired (*Directions for Making It.* – For every gallon of water put in three-quarters of a pound of alum (one pint) and a pound and three-quarters of salt (about one quart), and heat the liquid to the boiling-point, stirring occasionally, so that the salt and alum dissolve. Then pour it into a wooden, earthen, or glass vessel, or a tank lined with lead...and when it is cool, or even milk warm, it is ready for use. (p. 30))	(Hornaday 1912: 103)	13.5% w/v	1 week	Room temperature	J.M. Loveridge plc, BN A631
Ammonium hydroxide [1336-21-6]	water	Bone	Zoology	30%	24 hours		Skeleton preparation: soaked for 24 hours in 28-30% ammonium hydroxide solution, rinsed in distilled water, and air dried	(Hildebrand 1968 in Williams 1999: 71)	35%	24 hours	Room temperature	BDH, GPR 35% Ammonia solution, sp gr. 0.880, Prod: 27141, Lot: 5970160J, 35% NH3

Chemical tested [CAS number]	Solvent	Material treated	Collection type	Published concentration (%)	Published exposure time	Published temperature	Instructions and Comments	Reference	Experimental concentration (%)	Experimental exposure time	Experimental temperature	Chemical source information
Amyl acetate [628-63-7]				100%			Solvent: cited in: (Bather 1908: 87; British Museum (Natural History) 1934: 5; Camp and Hanna 1937: 31-32; Green 2001: 83 (referred to Rixon 1976); Koob 1982: 33; Lindsay 1987: 460 (referred to Rixon 1949); Nichols and Orr 1932: 49, 49-50; North et al. 1941: 77; Rixon 1976: 9; Satyamurti 1967: 16; Wagstaffe and Fidler 1968: 284, 285)	See Instructions and comments	100%	2 hours	Room temperature	Alfa Aesar, Technical Grade 100%, Prod: 036285, Lot: D15J08, EC: 211-047-3
Arsenic trioxide [1327-53-3]	water	Bone/skin/ fur	Zoology	3% w/v			Pesticide: Arsenic Water .... – Water, 4 quarts; arsenic, 4 ounces. Mix, stir and boil until the arsenic is all taken up. [Arsenic trioxide was used, as the only specific reference to the type of arsenic to use in any treatments in this reference was arsenic trioxide (p. 347)]	(Hornaday 1912: 348)	4.5% w/v	2 hours	Room temperature	Aldrich, 99%, Prod: 22, 762-5, Lot: S05309-302, CAS: 1327-53-3, EC: 215-481-4
Benzene [71-43-2]		Bone	Zoology	100%			Degreasing agent: Trichloroethylene is a widely used degreasing agent, but, because of its harmful characteristics, it is less used today. Benzene, chloroform and carbon tetrachloride are also dangerous substances...fat dissolving qualities are excellent. ...defatting may be carried out by immersing the specimens in any of the above solvents. The effect of the solvents is increased at a higher temperature	(Hangay and Dingley 1985: 345-347)	100%	1 week	Room temperature	BDH Laboratory Supplies, AnalaR, 99.7%, Prod: 100514E, Lot: K28372065 146, EC: 200-753-7
Carbon tetrachloride [56-23-5]		Bone	Zoology	100%	1 week +		Degreasing agent: [after boiling in potassium carbonate, ammonia and sodium sulfide solution, soak in warm water, dry] When dry, place them in carbon tetrachloride and allow them to soak a few days to degrease. A better and quicker way, but one which uses more carbon by loss through evaporation, is to place the bones in carbon tetrachloride in a double boiler and heat on the gas stove until the carbon boils, which will be at about 170F. Boil for fifteen minutes, or longer on thick bones, then set aside to cool off. ... sometimes mixed half and half with benzine.	(Rowley 1925: 211-213)	100%	1 week	Room temperature	Prolabo Normapur AR 99.8%, Prod: 22 521.293, Lot: L067, EC: 200-262-8

Chemical tested [CAS number]	Solvent	Material treated	Collection type	Published concentration (%)	Published exposure time	Published temperature	Instructions and Comments	Reference	Experimental concentration (%)	Experimental exposure time	Experimental temperature	Chemical source information
Cellulose nitrate [9004-70-0]	ether and alcohol (1:1)	Bone/keratin /antler	Zoology	~24.4-24.7%			Finishing material: Hendley's Enamel Varnish. – Take equal parts of ether and alcohol, mix them, and add one-third as much gun-cotton. To every gill of this mixture add six drops of olive-oil to give elasticity.	(Hornaday 1912: 346)	24.7%	1 hour	Room temperature	HMG (nitrocellulose) Heat and Waterproof Adhesive, Lot: 405064 (evaporated off solvent and redissolved in ether/ethanol)
1:1 Ethanol:ether		Bone/keratin /antler	Zoology				Finishing material: Hendley's Enamel Varnish. – Take equal parts of ether and alcohol, mix them, and add one-third as much gun-cotton. To every gill of this mixture add six drops of olive-oil to give elasticity.	(Hornaday 1912: 346)		1 hour	Room temperature	Ethanol as below; and ether: BDH AnalaR, Prod: 10094 6B, Lot: 331K19890127
Chloroform [67-66-3]		Bone	Zoology	100%	few hours		Degreasing agent: Suitable de-fatting agents are undiluted acetone, benzene, xylene or chloroform. A few hours immersion will suffice...	(Harris 1959: 223)	100%	overnight (18 hours)	Room temperature	BDH, AnalaR, Prod: 100775A, Lot: K32079841 323, EC: 200-663-8
Enzyme active detergent	water	Bone/skin/ fur	Zoology	10% v/v	30 min - 8 hours/day, 3-5 days	75-80°C	Skeleton preparation: immersion in 10% v/v solution, heated to 75-80°C; One fresh cat skull required only 30 minutes of simmering whereas formalin-fixed human skulls required 8 hours of simmering per day for 3-5 days; formalin fixed specimens take longer and do not bleach effectively; most detergents also contain sodium perborate, which bleaches the bone as it simmers	(Mooney et al. 1982: 125)	~9.9% w/v	40 hours	Room temperature and 80°C	Sainsbury's Basics Biological Powder, 5-15% phosphate, less than 5% oxygen-based bleaching agent, non-ionic surfactant, ionic surfactant, contains perfume, enzymes and optical brightener
Ethanol [64-17-5]		Skins/Fur	Zoology	6%	24 hours, repeat		Degreasing agent: immerse it in the following solution: gasoline two gallons, alcohol one pint, spirits of turpentine four ounces. The skins may be immersed in this solution for twenty-four hours, then squeezed out...until as much as possible of the grease is removed, then immerse the skin in a fresh solution made as above and rinse out thoroughly [rinse, dry and poison]	(Anderson 1932: 410-411)	99% v/v	48 hours	Room temperature	VWR International, BDH Anala R, Prod: 10107, EC: 200-578-6

Chemical tested [CAS number]	Solvent	Material treated	Collection type	Published concentration (%)	Published exposure time	Published temperature	Instructions and Comments	Reference	Experimental concentration (%)	Experimental exposure time	Experimental temperature	Chemical source information
Ethyl acetate [141-78-6]				100%			Solvent: cited in: (Bather 1908: 82-83 (referred to Reid, no date); Croucher and Woolley 1982: 46, 47; Jackson 1926: 117-118 (referred to Back 1924); Lindsay 1987: 460 (also referred to Bather 1908); Rixon 1976: 11; Whybrow and Lindsay 1990: 501)	See Instructions and comments	100%	2 hours	Room temperature	VWR International Ltd., AnalaR 99.5%, Prod: 101084H, Lot: K33783169 440, EC: 205-500-4
Ethylene diaminetetracetic acid, disodium salt (EDTA) [139-33-3 (anhydrous); 6381-92-6 (dihydrate)]	water	Ivory	Archaeology	5% w/v	6 weeks (fresh solution each week)		Chelating agent: Experiment - ivory was treated with disodium salt of EDTA (5% w/v), fresh solution for 6 weeks (500 ppm of benzalkonium chloride was added to each solution after the first week) found to be damaging	(Godfrey et al. 2002: 530)	7.5% w/v	1 week	Room temperature	GPR [VWR] International Ltd., Prod: 280254D, Lot: 0965T02265 340, EC: 205-358-3
Gasoline [8006-61-9]	alcohol/ turpentine	Skins/Fur	Zoology	93% v/v	24 hours, repeat		Degreasing agent: immerse it in the following solution: gasoline two gallons, alcohol one pint, spirits of turpentine four ounces. The skins may be immersed in this solution for twenty-four hours, then squeezed out...until as much as possible of the grease is removed, then immerse the skin in a fresh solution made as above and rinse out thoroughly [rinse, dry and poison]	(Anderson 1932: 410-411)	93% v/v	48 hours	Room temperature	97 Octane, unleaded
Gum arabic [9000-01-5]	water	Bone/Fossil	Palaeontology	50%			Consolidant: The gum water is made by dissolving lumps of commercial gum arabic, or the clear gum which exudes from the acacia (mimosa) tree, in an equal quantity of water; spray on	(Camp and Hanna 1937: 16-17)	75% w/v	1 hour	Room temperature	BDH Chemicals Ltd., Prod: 33001
Hydrogen peroxide [7722-84-1]		Bone	Zoology	20 volumes hydrogen peroxide	1 hour		Bleaching agent: [after skeleton maceration] Immersion in a solution of 20 volumes hydrogen peroxide with a trace of ammonium hydroxide for about an hour... the solution may be [re]used several times	(Harris 1959: 223)	9% v/v	1 hour	Room temperature	Bell's Hydrogen Peroxide Solution with stabilizer, 9% (30 volumes), Prod: BN5278F1

Chemical tested [CAS number]	Solvent	Material treated	Collection type	Published concentration (%)	Published exposure time	Published temperature	Instructions and Comments	Reference	Experimental concentration (%)	Experimental exposure time	Experimental temperature	Chemical source information
Industrial methylated spirit (IMS) [64-17-5]			Archaeology	100%	5 seconds, 4-5 times		Drying agent: [object] placed in a shallow earthenware dish, and covered with distilled water. This water was discarded and replaced with fresh distilled water at intervals of five seconds, four or five washings being...sufficient. ...immediately washed in 80 per cent. alcohol or industrial methylated spirit for a similar period. Finally, the object was immersed in ether for one minute, and dried in air. ...entire cleaning was completed within three minutes	(Plenderleith 1962: 148)	100%	25 seconds	Room temperature	Hayman Ltd., Batch: 6/D/339/26
Kerosene [8008-20-6]		Fossil	Palaeontology	100%			Cleaning agent: The fossil-bearing sediment is first thoroughly dried and then allowed to soak in kerosene in a container long enough to permit complete saturation. The sediment is then removed and placed in water, taken out, dried, and the released fossil finally given a coat of Glyptal	(Satyamurti 1967: 16)	100%	1 hour	Room temperature	Superwarm Fuel, Parasene, Kerosene, Paraffin
Linseed oil [8001-26-1]	turpentine	Keratin	Zoology	50%			Finishing material: Paint the legs and beaks of such birds as require it, with a mixture of boiled linseed oil and turpentine, equal parts of each, and have your paint thin enough on the legs that it will not obscure the scales. ... A little white wax softened and cut with turpentine and mixed with the paint on a bird's beak gives the color a depth...	(Hornaday 1912: 255)	50% v/v	2 hours	Room temperature	Homebase boiled linseed oil, Prod: K17015
Mercury (II) chloride [7487-94-7]	1:1 alcohol: water	Fur/skin	Zoology	saturated solution			Pesticide: If, [after cleaning]... the hair has not yet been poisoned, ... pour into the hair, so that it will run immediately down to the roots, a solution of alcohol, water, and corrosive sublimate made as follows: If you wish to make four gallons of the solution, take two gallons of ninety-five per cent. Alcohol, dissolve it in all the corrosive sublimate it will take up, making...a "saturated solution." ...Carefully pour off the clear liquid so as to leave the sediment remaining in the jar, and then dilute the former with an equal quantity of water ...In case the solution used should leave a gray deposit on the hair, it should be sponged off with a little warm water	(Hornaday 1912: 150-151)	~7.5% w/v	2 hours	Room temperature	Aldrich, Prod: 215465-5G, Batch: 03706DO

Chemical tested [CAS number]	Solvent	Material treated	Collection type	Published concentration (%)	Published exposure time	Published temperature	Instructions and Comments	Reference	Experimental concentration (%)	Experimental exposure time	Experimental temperature	Chemical source information
Methylmethacrylate/ethylacrylate resin [80-62-6]	acetone	Bone	Archaeology	30%	overnight		Consolidant: A 30-percent solution of Acryloid B-72 and acetone was prepared as a consolidant. This solution was brushed on the inferior surfaces first, then allowed to dry overnight. The consolidant was then brushed on the superior surface. Only one coat was applied	(Cannon 1997: 36)	30% v/v	overnight (18 hours)	Room temperature	Conservation Resources, Paraloid B-72
Oxalic acid [144-62-7 (anhydrous); 6153-56-6 (dihydrate)]	water	Bone	Archaeology	10% w/v			Chelating agent: 5-10% oxalic acid can be used to remove iron stains; rinse and consolidate with PVA or B-72 after	(Hamilton 1999/2001: 314-315)	15% w/v	1 week	Room temperature	no data available
Pepsin [9001-75-6]	water	Bone	Zoology	1% w/v	up to 48 hours	37°C	Skeleton preparation: 1% trypsin *(pepsin)* in 0.5% aqueous sodium carbonate, up to 48 hours at 37°C, rinse and bleach for 24 hours	(Mahoney 1973: 442) (Hangay and Dingley 1985: 344 note that pepsin and trypsin are used under similar conditions)	1.5% w/v	48 hours	Room temperature and 37°C	Sigma, (Pepsin A/ 800-2,500 units/mg protein, EC 3.4.23.1), 1:10,000, P-7000, Lot: 103K0099, From Porcine stomach mucosa [9001-75-6], EC: 232-629-3
Poly(vinyl) acetate (PVAC) [9003-20-7]	water	Fossil/Bone	Palaeontology	1/3 dilution	6-12 hours		Consolidant: ...immerse in an aqueous emulsion of polyvinyl acetate. The strength required is about one third of that supplied by the makers. This impregnation may be done in vacuo, but where vacuum equipment is not available an immersion of from six to twelve hours will ensure adequate penetration	(Toombs and Rixon 1950: 141)	49.5% v/v	overnight (18 hours)	Room temperature	Brian Clegg Washable P.V.A. Adhesive
Poly(vinyl) acetate/poly(vinyl) alcohol emulsion (PVAC/PVAL)	water	Bone	Anthropology	20%			Consolidant: The glue [Elmer's] should be made up about 1/5 to 1/6 of the mixture. ...Longer bones will have to be dipped one end at a time. Large or porous bones need to be submerged until air bubbles no longer come to the surface. ...If [sic] may be desirable to redip some bones after they have dried from the first dipping	(Lewis and Redfield 1970: 7-8)	30% v/v	overnight (18 hours)	Room temperature	Elmer's Glue-All, Elmer's Products Inc.

Chemical tested [CAS number]	Solvent	Material treated	Collection type	Published concentration (%)	Published exposure time	Published temperature	Instructions and Comments	Reference	Experimental concentration (%)	Experimental exposure time	Experimental temperature	Chemical source information
Poly(vinyl) butyral resin [63148-65-2]	9:1 IMS: diacetone alcohol (1:1 IMS :acetone)	Bone	Palaeontology	20% w/v			Consolidant: 20% w/v in acetone, may be applied over B98; another author suggests mixing poly(vinyl) butyral resins with alcohol and acetone (Green 2001: 247).	(Croucher and Woolley 1982: 28)	20% w/v	1 hour	Room temperature	Conservation Resources, Mowital B30H
1:1 Acetone:IMS							Solvent: (see poly(vinyl) butyral resin)	see poly(vinyl) butyral resin		1 hour	Room temperature	Acetone and IMS as above
Potassium carbonate (potash) [584-08-7]	water	Skins	Zoology	3% w/v		simmer	Cleaning agent: bones can be simmered in a solution of 3 oz potassium carbonate and 1 ½ oz sodium sulphide in 5 pints of water, in a glass or enamel vessel. ...Any sulphide smell retained by the bones can be destroyed by a dilute ammonia solution	(Ryder 1968: 23)	4.5% w/v	6 hours	Room temperature and 80°C	BDH Laboratory Supplies, AnalaR, Potassium carbonate anhydrous, Prod: 101964H, Lot: A919836 636, EEC: 209-529-3
Shellac [9000-59-3]	alcohol	Bone	Palaeontology	50%			Consolidant: Shellac should be "pure white," not orange nor compound. It must be thinned (1/2 to 2/3) with alcohol. Fresh shellac requires a dilution of half-and-half	(Camp and Hanna 1937: 9-10)	50%	1 hour	Room temperature	L. Cornelissen & Son, clear dewaxed shellac
Sodium bicarbonate [144-55-8]	water	Bone	Zoology	10% w/v	12-24 hours	80°C	Degreasing agent: after maceration the bones are immersed in clean water and 5%-10% sodium bicarbonate is added. The water is brought to 80°C and kept at this temperature for 12 to 24 hours. ... As soon as softening of the bone surface occurs, the specimens must be removed from the solution, rinsed in clean water, and dried. Liquid detergent added to the hot water maceration process will also aid degreasing to a certain extent; least dangerous	(Hangay and Dingley 1985: 347)	15% w/v	24 hours	Room temperature and 80°C	Super Cook Bicarbonate of Soda

Chemical tested [CAS number]	Solvent	Material treated	Collection type	Published concentration (%)	Published exposure time	Published temperature	Instructions and Comments	Reference	Experimental concentration (%)	Experimental exposure time	Experimental temperature	Chemical source information
Sodium carbonate (washing soda) [497-19-8]	water	Bone	Zoology	10% w/v	5 min		Degreasing agent: every ligamentary skeleton must be dried before it is finally cleaned and mounted. ...the skeleton is soaked in clear water for two or three days, or longer as may be necessary...While a small skeleton is undergoing the scraping process it must not be allowed to get dry until it is finally set up in position. When the skeleton is not being worked upon, it must be kept soaking in clean water... A little borax in the water serves to arrest decomposition, and will allow a skeleton to remain soaking for several days longer than could otherwise be allowed. After a skeleton has been well scraped, in order to get it as white as possible and free from grease, it must be treated with Javelle water: ½ pound chloride of lime, 1 pound common washing soda, 1 gallon of boiling water. Keep this on hand...in the dark. ...draw off a small quantity in a broad, shallow, earthen dish. Lay every small skeleton in it, and with a soft tooth-brush...brush all the bones thoroughly for about 5 minutes. ...wash the skeleton thoroughly with clear water, and perhaps it is then ready to mount.	(Hornaday 1912: 285-288)	15% w/v	5 minutes	Room temperature	BDH Laboratory Supplies, GPR, Prod: 301214L, Lot: A217831 016
Sodium chloride (salt) [7647-14-5]	water	Skins/Fur	Zoology	0.9% w/v	24 hours	up to 50°C	Skeleton preparation: (Mammal saline is 0.9 per cent NaCl in distilled water (p. 223)) place specimen in saline and add papaine at a rate of 0.5 grm per 100 ccs saline. The resultant fluid is then incubated for 24 hours. Solution may be used over again with good and quicker results at higher temperatures (up to 50°C). Will not need degreasing, but can be bleached	(Harris 1959: 223-224)	1.35% w/v	24 hours	Room temperature and 37°C	VWR International Ltd., GPR, Prod: 301235Q, Lot: K34042132
Sodium hydroxide [1310-73-2]	water	Bone	Zoology	1%	3-4 hours repeated for several days		Skeleton preparation: Stephens ('79), uses household bleach (sodium hypochlorite, 4.5% to 6%). Sodium hydroxide, 5 to 10 g/liter of diluted bleach solution, is also required. It is primarily suggested for use on decomposed remains, requiring only defatting and drying. The bleach is for cleaning away soft tissue. For defatting, acetone (purer grade) is suggested.	(Stephens 1979 in Krogman and Işçan 1986: 42)	1.5% w/v	28 hours	Room temperature	BDH Laboratory Supplies, AnalaR, min 99%, Prod: 102524X, Lot: B868650 124, EC: 215-185-5

Chemical tested [CAS number]	Solvent	Material treated	Collection type	Published concentration (%)	Published exposure time	Published temperature	Instructions and Comments	Reference	Experimental concentration (%)	Experimental exposure time	Experimental temperature	Chemical source information
Sodium hypochlorite (bleach) [7681-52-9]		Bone	Zoology	6%	3-4 hours repeated for several days		Skeleton preparation: Stephens ('79), uses household bleach (sodium hypochlorite, 4.5% to 6%). Sodium hydroxide, 5 to 10 g/liter of diluted bleach solution, is also required. It is primarily suggested for use on decomposed remains, requiring only defatting and drying. The bleach is for cleaning away soft tissue. For defatting, acetone (purer grade) is suggested.	(Stephens 1979 in Krogman and Işçan 1986: 42)	9% v/v	28 hours	Room temperature	BDH Laboratory Supplies, GPR, ≥12% available chlorine, Prod: 301696S Lot: K31848623 317
Sodium perborate [7632-04-4]	water	Bone	Zoology	10% w/v	overnight	from boiling	Skeleton preparation: Sodium perborate (60g - 70g for smaller specimens; 70g-100g for larger specimens) is added to each litre of boiling water. The perborate is dissolved in the hot water and the bones are then immersed in it. The container is covered and allowed to cool. [soak overnight then clean]	(Roche 1954 translated by Hangay and Dingley 1985: 342-343)	15% w/v	overnight (18 hours)	Room temperature and heated to 100°C for 1 minute in PCR machine then allowed to cool to room temperature	BDH Limited, GPR, Min. assay 96.0%, Prod: 30196, Lot: 3367530M
Sodium sulfide [1313-82-2]	water (saline)	Bone	Zoology	0.1% w/v	6 hours	simmer	Skeleton preparation: A modification of Rowley's Fluid (sodium sulphide 1 grm., pancreatin 2 grms., and saline (0.9%) 1 litre) was prepared and the specimen selected gently simmered for 6 hours. ...Papaine incubation maceration was commenced. A 1 per cent saline solution of papaine containing the specimen was incubated for 12 hours at 37°C. The solution was then removed from the incubator, brought slowly to the boil, and allowed to simmer for 1 hour. The bones were bleached in a solution of 10 volumes hydrogen peroxide with a trace of ammonia, and then dried in acetone, to complete the preparation	(Harris 1951: 97)	0.15% w/v	6 hours	Room temperature and 80°C	Alfa Aesar, Johnson Matthey GmbH & Co., hydrate: 27610-45-3, Prod: 011664, Lot: C11Q49, EC: 215-211-5

Chemical tested [CAS number]	Solvent	Material treated	Collection type	Published concentration (%)	Published exposure time	Published temperature	Instructions and Comments	Reference	Experimental concentration (%)	Experimental exposure time	Experimental temperature	Chemical source information
Toluene [108-88-3]				100%			Solvent: cited in: (Cornwall 1956: 213, 214, 216-217; Davidson 2004: 55; Gehlert 1980: 8; Green 2001: 83 (referred to Rixon 1976); Horie 1987: 96 (referred to Unwin 1951); Howie 1979: 280; Johnson 1994: 227 (referred to Brothwell 1981), 229; Koob 1984: 100; Kres and Lovell 1995: 510; Leigh 1978: 33; Mahoney 1973: 449; North et al. 1941: 78; Payton 1992: 23; Rixon 1976: 10, 11; Satyamurti 1967: 16; Sease 1994: 51; Shelton and Johnson 1995: 66; Snow and Weisser 1984: 142; Storch 2003: 4; Thurmond 1974: 195)	See Instructions and comments	100%	2 hours	Room temperature	BDH Laboratory Supplies, GPR, Prod: 30454EC, EC: 203-625-9
Trichloroethylene [79-01-6]		Bone	Zoology	100%			Degreasing agent: immerse the bones in a large quantity of trichloroethylene, or in a mixture of benzene and chloroform. By far the best method is to reflux the trichloroethylene and to place the bones in the vapour, allowing the fat to drip away into the reservoir of liquid solvent. ... best grade of trichloroethylene is 'Triklone' N (trichloroethylene, Grade 7)...	(Mahoney 1973: 445)	100%	2 hours	Room temperature	Fluka Chemika, Prod: 91129, Lot: 431827/142202, EC: 2011674
Turpentine [8006-64-2]		Skins/Fur	Zoology	1%	24 hours, repeat		Degreasing agent: immerse it in the following solution: gasoline two gallons, alcohol one pint, spirits of turpentine four ounces. The skins may be immersed in this solution for twenty-four hours, then squeezed out...until as much as possible of the grease is removed, then immerse the skin in a fresh solution made as above and rinse out thoroughly [rinse, dry and poison]	(Anderson 1932: 410-411)	100%	48 hours	Room temperature	Hilton Banks Ltd., spirits of turpentine, EC: 232-350-7
White spirit, (naptha) [8052-41-3]		Bone	Zoology	100%	several days		Degreasing agent: Often the bones of a small skeleton contain an inordinate amount of grease. The easiest and simplest way to remove it is to soak the greasy bones for several days or weeks, as may be necessary, in a jar of pure naphtha.	(Hornaday 1912: 287-288)	100%	1 week	Room temperature	Prolabo, Prod: 28 963.368, Lot: K243, EC: 232-443-2
Xylene [1330-20-7]		Bone	Zoology	100%	few hours		Degreasing agent: Suitable de-fatting agents are undiluted acetone, benzene, xylene or chloroform. A few hours immersion will suffice...	(Harris 1959: 223)	100%	overnight (18 hours)	Room temperature	BDH Laboratory Supplies, GPR, 99.0%, Prod: 305756G, Lot: K31502911 250, EC: 215-535-7

Table 7. Chemicals tested in the screening experiment, their published and experimental conditions and instructions, as well as source information. The language of the original reference was preserved as much as possible. "Prod:" = product number, "Lot:" = lot number, "EC:" = European Commission number.

4.1.2. PCR conditions and primers

PCR products were used as a source of DNA rather than archaeological or other specimen material to screen chemicals used in the past in preparation or conservation treatments for their effects on DNA. This was done because the initial condition and amount of DNA in each sample could be accounted for, enabling comparison across samples to identify those treatments damaging to DNA as well as treatments that may promote DNA preservation. PCR products were generated of sequences routinely used in the laboratory where this work was carried out, because source DNA for the target sequences was readily available and previously established methods for generating and analysing these sequences could be adapted for this study.

The PCR conditions and primers used for this research are modifications of those published by Thomas et al. (1999). Four PCR products were generated for this study, two of which were around 100bp in length (YAP- and TAT), and another two around 200bp in length (M9 and SRY 4064). The sequences of the primers used are listed in Table 8.

Primer name	Primer sequence (5' - 3')	PCR product name	Length (bp)
YAP-C-NED	NED-AGG ACT AGC AAT AGC AGG GGA AGA	YAP-	99
YAP-D-Biotin	BIO-CAG GGC CAA CTC CAA CCA AG		
Tat-R-NED	NED-GAA GGT GCC GTA AAA GTG TGA A	TAT	112
Tat-L-Biotin	BIO-GAC TCT GAG TGT AGA CTT GTG A		
M9-L-NED	NED-TCA GGA CCC TGA AAT ACA GAA CT	M9	214
M9-R-Biotin	BIO-TTG AAG CTC GTG AAA CAG AAT AG		
SRY4064-L-NED	NED-GGT ATG ACA GGG GAT GAT GTG A	SRY 4064	225
SRY4064-R	CCA CGC CCA GCT AAT TTT TTG T		

Table 8. Primers and primer sequences used in this study, as well as PCR product abbreviation and length of the PCR product.

Some primers were biotinylated, as a method using Dynabeads® was originally conceived to quantify DNA loss, but preliminary experiments indicated a lack of adequate binding, and this method was abandoned. However, since biotin would not affect fluorescence of PCR products, unbiotinylated primers were not necessary.

TAT and M9 PCR amplifications were performed separately in final reaction volumes of 25 µl, consisting of 1 µl of gel purified PCR products from a previous PCR as template DNA (from an anonymous source known to have the target sequence), 250 µM of dNTPs, 0.1 units of SuperTaq™ polymerase (Enzyme Technologies Limited), 2.5 µl of the 10 x buffer supplied with the SuperTaq™, and 0.64 µM of each primer. YAP- and SRY 4064 amplifications were performed separately as above, except 0.32 µM of each primer was used.

Cycling parameters for TAT and M9 amplification were pre-incubation for 5 min at 95°C, followed by 38 cycles of 1 min at 94°C for denaturation, 1 min at 55°C for annealing, 1 min at

72°C for extension, and then a final incubation for 10 min at 72°C. TAT and M9 amplifications were performed in a DNA Engine DYAD Peltier Thermal Cycler.

Cycling parameters for YAP- and SRY 4064 amplifications were pre-incubation for 4 min at 95°C, followed by 40 cycles of 1 min at 94°C for denaturation, 1 min at 59°C for annealing, 1 min 40 sec at 72°C for extension, and then a final incubation for 10 min at 72°C. YAP- and SRY 4064 amplifications performed in on a Biometra UNO II Peltier Thermal Cycler.

The resulting PCR products for each sequence were then pooled and purified using a Vivaspin 30K molecular weight cut-off (MWCO) centrifugal ultrafiltration column (Vivascience, UK). Columns were washed three times with TE buffer (10 mM Tris-HCl pH 8.0, 1 mM EDTA), and retained DNA resuspended in 500 µl TE buffer.

4.1.3. Experimental protocol

A two-fold series dilution was set up for each of the purified PCR products, which was analysed using capillary electrophoresis on an ABI PRISM® 3100 Genetic Analyzer under the same conditions and settings future test samples would be analysed (see below). Data were analysed using ABI PRISM® GeneScan® Analysis software version 3.7 for Windows (Applied Biosystems), and PCR products were diluted to fall within the detection limits of the equipment used based upon assessment of the peak height data gathered. Diluted PCR products were mixed to create two solutions. A "test" stock was made, to which treatment solutions were added. A "standard" stock was also made, which was added to the combined test stock and chemical treatment solution after the experimental treatment period ended. Each stock solution contained one 100bp fragment and one 200bp fragment. By calculating the ratio of the peak heights between the 100bp fragment and the 200bp fragment in the test stock and the 100bp fragment and the 200bp fragment in the standard stock, damage to the test stock could be assessed, as any damage to DNA resulting from sample preparation procedures after chemical treatment should affect both the test and standard stocks equally. The ratio between the test stock and standard stock, therefore, reflects only damage to the DNA fragments in the test stock resulting solely during the chemical treatment, negating any potential loss of DNA resulting from subsequent sample preparation procedures.

TAT and SRY 4064 PCR products were mixed to create the test stock to be treated, consisting of: 90.9 µl TAT purified PCR products, 284.1 µl SRY 4064 PCR products, 5284.1 µl 0.1 x TE buffer and 22.7 µl 5% xylene cyanole blue in sterile water. YAP- and M9 PCR products were mixed to create the standard stock consisting of: 170.5 µl M9 PCR products, 284.1 µl YAP- PCR products, 5204.5 µl 0.1 x TE buffer and 22.7 µl 5% xylene cyanole blue in sterile water. Xylene cyanole blue was added to the stocks to enable better visibility of the aqueous phase during ether extraction of the organic solvent treatment solutions. Although xylene cyanole

blue was only necessary in the test stock which was ether extracted, it was added to both stocks at the same concentration so any possible interaction with the DNA would be equal across all samples.

For each conservation treatment sample, 15 µl of test stock was loaded into a 96-well PCR plate. Treatment solutions were added in 30 µl volumes at the appropriate exposure time, so that all treatments concluded simultaneously. Organic solvent-based treatment samples had an additional 30 µl of water added at the outset to maintain equal water volumes across all samples. In addition to 44 treatments tested, two water controls and two mineral oil samples were run in each treatment set, as well as an additional 8 treatment controls and 8 mineral oil samples. Four replicate sets of each treatment were run, and each sample was analysed twice to ensure consistency across samples for each treatment.

Heated samples were maintained at the required temperature in ovens. For each heated treatment, unheated replicate samples of the same treatment were also set up. This was done to better account for the effects of the chemical treatment and of heating the treatment separately. To ensure contact between the test stock and treatment solutions, unheated samples were continuously mixed on a Vortex Genie 2® at low speed for the duration of the prescribed exposure time (heated treatments could not be mixed). Experimental exposure time refers to the time the treatment was in contact with the DNA on a mixer. At the end of treatment exposure time, heated samples were removed from the ovens and all aqueous samples were stored at 4°C whilst organic solvent-based samples underwent ether extraction. MilliQ water was added to heated treatments to compensate for any loss by evaporation. Ether extraction was carried out on all organic solvent-based treatments by adding 170 µl of ether to the sample, mixing, and pipetting off the organic solvent phase three times, followed by air drying for 10 minutes. Then, 15 µl of standard stock was added to all samples. Samples were frozen overnight prior to precipitation to minimise further DNA damage from treatment solutions or residues.

Following initial experiments to establish the most effective sample precipitation and preparation method (isopropanol precipitation or a MicroClean based clean up procedure), an isopropanol precipitation and sample preparation protocol was devised as follows:

1. To each sample consisting of 60 µl combined volume of test and standard stock, add 20 µl of 0.8M NaCl to each tube

2. Add 170 µl of chilled 99% isopropanol to each tube

3. Mix thoroughly and incubate at room temperature for 10 minutes

4. Centrifuge at 4000 rpm for 40 minutes

5. Centrifuge upside down at 300 rpm for 15 sec to remove supernatant

6. Add 150 µl of chilled 70% ethanol to each tube

7. Centrifuge at 4000 rpm for 20 min

8. Centrifuge upside-down at 300 rpm for 15 sec to remove supernatant

9. Dry for 5 min at 65C in PCR machine

10. Add 10 µl TE buffer to resuspend DNA

11. Freeze until needed

12. Thaw, mix and centrifuge briefly to spin down

13. Remove 1 µl of sample to a 96-well plate, and add 10 µl internal size standard mixed with deionised formamide (1:90 GeneScan™-500 ROX™:Hi-Di™ formamide (both Applied Biosystems)) to each tube

14. Heat to 96°C for 4 min and snap-cool on ice

15. Run samples under denaturing conditions using capillary electrophoresis on an ABI PRISM® 3100 POP-6 polymer in a 50 cm capillary array on an ABI PRISM® 3100 Genetic Analyzer, at 60°C, for 4000 seconds, using filter set D. Analyse data using ABI PRISM® GeneScan® Analysis software version 3.7 for Windows (Applied Biosystems).

Aqueous treatment stock solutions had to be mixed at a concentration 50% higher than the published concentration, which approached or exceeded the solubility of a few compounds in water. Furthermore, the final concentration of some compounds exceeded their solubility in alcohol. This was considered potentially problematic, as removal of treatment chemicals was to be performed using isopropanol and ethanol, and co-precipitation of chemicals with the DNA could result in DNA pellet loss during centrifugation. Chemical residues could also ionically compete with the DNA during electrophoresis, or precipitation of treatment chemicals during sample processing could trap the DNA in crystals, thus hampering results. Alum, detergent,

EDTA, oxalic acid, sodium bicarbonate and sodium perborate were all mixed at concentrations exceeding their solubility limits under the conditions of this study. Diluted stock solutions were mixed of each of these chemicals at 2.5%, 5%, 7.5%, etc. (up to the concentration required for the experiment) and taken through the sample preparation protocol to determine if a lower concentration of any of these chemicals could be used, possibly at a longer exposure time to compensate for the dilution. However, even at the lowest concentration, all of the samples exhibited either precipitation or chemical residues. Therefore, for the screening experiment, all chemicals were used undiluted (meaning, at 50% greater concentration than that published), and additional clean up measures were tested to overcome co-precipitation problems.

After initial sample processing and analysis, in an attempt to further reduce or remove chemical residues and precipitates, two samples each of alum, detergent, EDTA, gum arabic, mercury (II) chloride, oxalic acid, sodium bicarbonate and sodium perborate were subjected to re-precipitation with isopropanol and ethanol. Each sample, consisting of approximately 8 µl liquid plus residue and/or precipitate, was resuspended in 60 µl of TE buffer, and the above sample preparation protocol was carried out again, with 4 washes of 200 µl 70% ethanol (i.e. steps 6-8 were repeated 4 times using a volume of 200 µl). This resulted in little change to the amount of residue or precipitate observed in the samples. Samples were reanalysed and data collected. These samples are referred to by chemical treatment name, followed by "re-ppt" in the results section (e.g. Alum – re-ppt).

In a further effort to remove low-solubility chemical precipitates, an additional single sample of alum, oxalic acid, sodium perborate and a water control sample were subjected to additional cleaning using a VivaSpin 500 10K MWCO ultrafiltration column (Vivascience). The column was first washed with 500 µl of TE buffer. Samples consisting of approximately 8 µl liquid plus residue and/or precipitate were resuspended in 200 µl of TE, resulting in dissolution of all precipitate, and loaded into the column. The original sample tubes were washed out with another 200 µl of TE which was added to the resuspended sample in the column. Samples were centrifuged at 10,000 rpm for approximately 5 minutes. Samples were washed with 500 µl TE a further three times. The final volume collected of eluate was between 5 and 8 µl. Samples were reanalysed and data collected. These samples are referred to by chemical treatment name, followed by "column cleaned" in the results section (e.g. Alum – column cleaned).

To confirm some initial results, particularly a lack of expected peaks or low peaks in some electropherograms, some treatments were repeated a second time. This was also done to confirm that freezing samples prior to precipitation and clean up had no effect on electrophoresis data, as the first lot of samples were frozen prior to precipitation and CE, but the repeated samples were frozen only after the first CE run. In order to repeat some treatments, a

second set of DNA test and standard stocks were required. These were mixed at the same concentration as before, but in a smaller volume. TAT and SRY 4064 PCR products were mixed to create a test stock to be treated, consisting of: 26.7 µl TAT purified PCR products, 83.3 µl SRY 4064 PCR products, 1550.0 µl 0.1 x TE buffer and 6.7 µl 5% xylene cyanole blue in sterile water. YAP- and M9 PCR products were mixed to create a standard stock, consisting of: 50.0 µl M9 PCR products, 83.3 µl YAP- PCR products, 1526.7 µl 0.1 x TE buffer and 6.7 µl 5% xylene cyanole blue in sterile water. The following treatments were repeated a second time: alum, detergent (heated and unheated), EDTA, mercury (II) chloride, oxalic acid, pepsin (heated and unheated), sodium bicarbonate (heated and unheated), sodium hypochlorite, sodium perborate (heated and unheated). Additional heated control samples were also run. The same chemical treatment stocks were used for the repeated samples, with the exception of enzyme-based treatments, namely enzyme active detergent and pepsin, for which new stocks were mixed. Repeated treatment samples using the second set of DNA stocks are referred to by chemical treatment name followed by (2) in the results section (e.g. Alum (2)).

4.1.4. Data analysis

Four PCR products were generated for use in this study, and the peaks produced by capillary electrophoresis were initially identified by running the test and standard stocks separately through the ABI PRISM® 3100, and looking for peaks representing fragments of the appropriate length (size), see Figure 3. PCR products were fluorescently-labelled with the dye NED, which fluoresces yellow, but is displayed as black peaks in the electropherograms for greater visibility against the white background. An internal size standard containing several fragments of known length was also included in each sample against which the PCR products were measured and their size (length in basepairs) determined. The internal size standard used was GeneScan™-500 ROX™ Size Standard (Applied Biosystems), which fluoresces red, and appears in the electropherograms as red peaks. Each peak represents an individual fragment. As voltage is applied, DNA fragments migrate through the capillaries at a rate dependant upon their length (shorter fragments travel faster than longer fragments), and pass across a laser beam. Peaks visualised on the electropherograms represent the amount of fluorescence recorded when dye-labelled fragments pass through the laser during electrophoresis, which is measured in relative fluorescent units (RFU), and equates to the relative concentration of each fragment. Some of the DNA fragments used in this study appear in electropherograms as multiple peaks (YAP = 5 peaks, M9 and SRY 4064 = 2 peaks each), due to mechanisms inherent in PCR amplification. As the DNA fragments used in this project are routinely studied in the laboratory where this research was performed, it is known that the configuration presented is considered normal (Thomas 2006 pers. comm.). Therefore, throughout this research, peaks are referred to by the sequence represented, and multiple peaks from a single sequence were differentiated by the addition of a letter, beginning with A, from shortest to longest strand represented (e.g. YAP A peak, YAP B peak, etc).

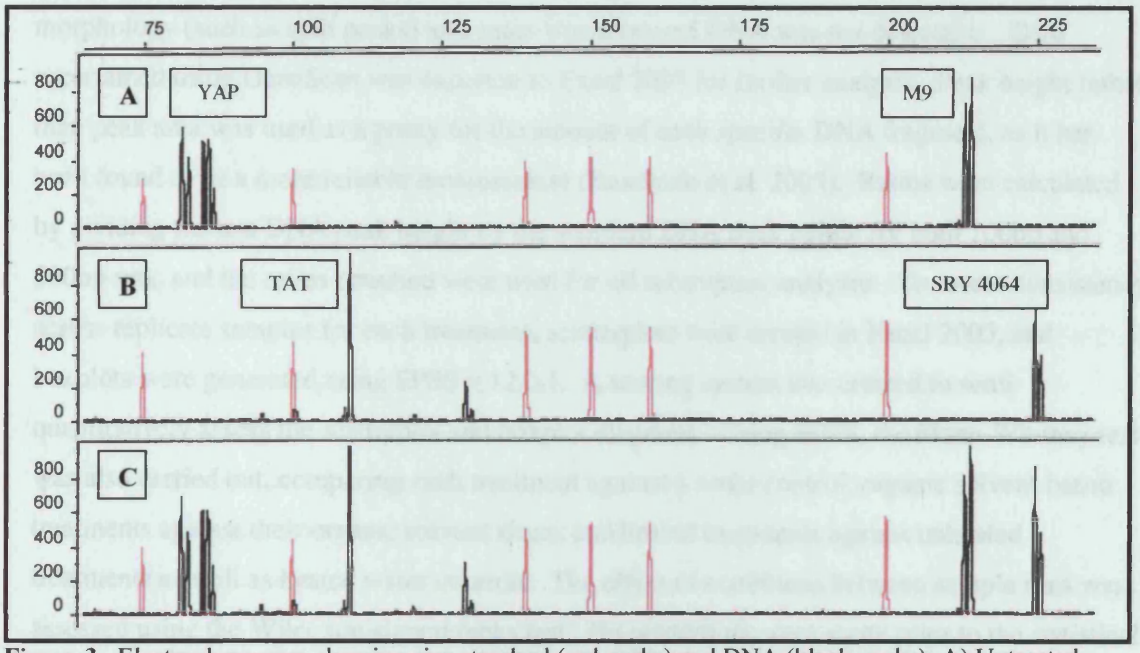


Figure 3. Electropherogram showing size standard (red peaks) and DNA (black peaks): A) Untreated standard stock, consisting of 5 YAP peaks and 2 M9 peaks, B) Treated stock, consisting of a single TAT peak and 2 SRY 4064 peaks, and C) Untreated and treated stock together, as an example of undamaged stocks.

Data were analysed using ABI PRISM® GeneScan® Analysis software version 3.7 for Windows (Applied Biosystems). Analysed samples had the following data recorded: minute, size, peak height, peak area and data point. Size, as well as the peak pattern, was used to identify each DNA fragment (confirmed by minute and data point if necessary).

Since this system determines the relative concentration of the DNA fragments in each sample rather than the absolute concentration, the ratio between the test and standard stock the peak heights for each sample was calculated and compared to the peak height ratios of control samples as a means of measuring damage. Ratios were calculated by dividing the test peak height by the standard peak height for both the 100bp (TAT/YAP-) and 200bp (SRY 4064/M9) sequence pairs. A smaller test peak height resulted in a lower ratio, thereby indicating damaged DNA, whereas a higher ratio indicates better preserved DNA. Ratios between test and standard stocks are referred to by the first letter of the conservation stock, the first letter of the standard stock followed by the peak letter (e.g. TYA, TYB, SMA, SMB, etc). Number 1 or 2 follows the ratio abbreviation to denote if the data represented are from the first or second run of the sample (e.g. TYA1, TYA2, etc). As the TAT sequence produced only a single peak, its data were repeated to calculate ratios with all each of the five YAP peaks (i.e. the TAT A peak data is the same as the TAT B peak, TAT C peak, etc).

The effects of the chemical treatments administered to short strands of DNA were assessed by qualitative, semi-quantitative, quantitative and statistical methods. Damage was assessed in the

first instance by visual inspection of electropherograms by identifying irregularities in peak morphology (such as split peaks) and cases where treated DNA was not detectable. Data ascertained using GeneScan was exported to Excel 2003 for further analysis. Peak height rather than peak area was used as a proxy for the amount of each specific DNA fragment, as it has been found to be a more reliable measurement (Baudhuin et al. 2005). Ratios were calculated by dividing the test DNA peak height by the standard DNA peak height for both 100bp and 200bp sets, and the ratios obtained were used for all subsequent analyses. To assess consistency across replicate samples for each treatment, scatterplots were created in Excel 2003, and boxplots were generated using SPSS v.12.0.1. A scoring system was created to semi-quantitatively assess the scatterplot and boxplot diagrams. Using SPSS, the Mann-Whitney test was also carried out, comparing each treatment against a water control, organic solvent-based treatments against their organic solvent alone, and heated treatments against unheated treatments as well as heated water controls. The effect of conditions between sample runs was assessed using the Wilcoxon signed ranks test. No predictions were made prior to the statistical analyses as to what effects the treatments tested would have, as previous predictive models based on nucleic acid chemistry (Brown 1999) were inconsistent with other published experimental results (Kigawa et al. 2003; Williams 1999), so two-tailed tests for significance were used. Peak height ratios were also used to calculate the percentage of DNA preservation to quantitatively assess the effect of each treatment tested.

4.2. Mummy case study

There is much debate surrounding the utility of ancient Egyptian mummies for DNA analyses (Gilbert et al. 2005b; Zink and Nerlich 2003; Zink and Nerlich 2005). There is also a renewed interest in identifying the materials used by ancient Egyptians in the mummification process (Buckley et al. 2004; Kaup et al. 2003; Koller et al. 2003; Koller et al. 2005; Tchapla et al. 2004; Weser and Kaup 2002). To assess the effects of ancient Egyptian mummification materials and methods on DNA, a case study was designed using hair from both ancient Egyptian cat mummies as well as a modern (approximately 5 years old) rabbit mummy produced using ancient Egyptian mummification materials and methods, as currently understood (Ikram 2005). Ancient Egyptian mummification methods may be considered as one of the first preparation and conservation treatments. The short term effects of basic mummification using alcohol and "natron" were investigated. Natron is technically sodium carbonate decahydrate ($Na_2CO_3 \cdot 10H_2O$) (Mullin 1993), but most "natron" from Wadi Natrun is a mixture of evaporitic salts, a high proportion of which are sodium carbonates, particularly sodium carbonate bicarbonate 2-hydrate ($Na_2CO_3 \cdot NaHCO_3 \cdot 2H_2O$), also known as trona (Shortland 2004: 499). The materials used in the experimental mummification process are closely related to chemicals assessed for their effects on DNA in the screening test of this study, namely ethanol, sodium carbonate and sodium bicarbonate.

Animal mummies were chosen for this study rather than human mummies to minimise the potential for contamination with modern DNA. Big cat bone samples had previously been processed in the laboratory (Barnett et al. 2005; 2006), so all facilities and equipment used had undergone thorough bleaching and UV treatment to eliminate any sources of contamination. Rabbit samples had never been processed in the laboratory prior to this study.

Hair was chosen for this research, as it is known to have a higher proportion of mitochondrial DNA (Gilbert et al. 2004) than other tissues, which should increase the possibility of containing amplifiable DNA. Hair samples from archaeological specimens have previously been used successfully for DNA analysis (Bonnichsen et al. 2001). It was also thought that hair DNA should be less affected by degradation in the short term, as the rapid desiccation and high salt content of the mummification conditions should minimise degradation by nucleases immediately after death (Hofreiter et al. 2001a). However, it is acknowledged that superficial tissues such as hair would also be subject to the greatest exposure to environmental conditions and conservation treatments in the long term, which could affect DNA preservation.

DNA from the hair samples was extracted, amplified by PCR, purified, cloned and sequenced using established protocols. In addition, the sensitivity of both the extraction procedure and the amplification conditions were quantified. For a generalised overview of the methods used, see Figure 4.

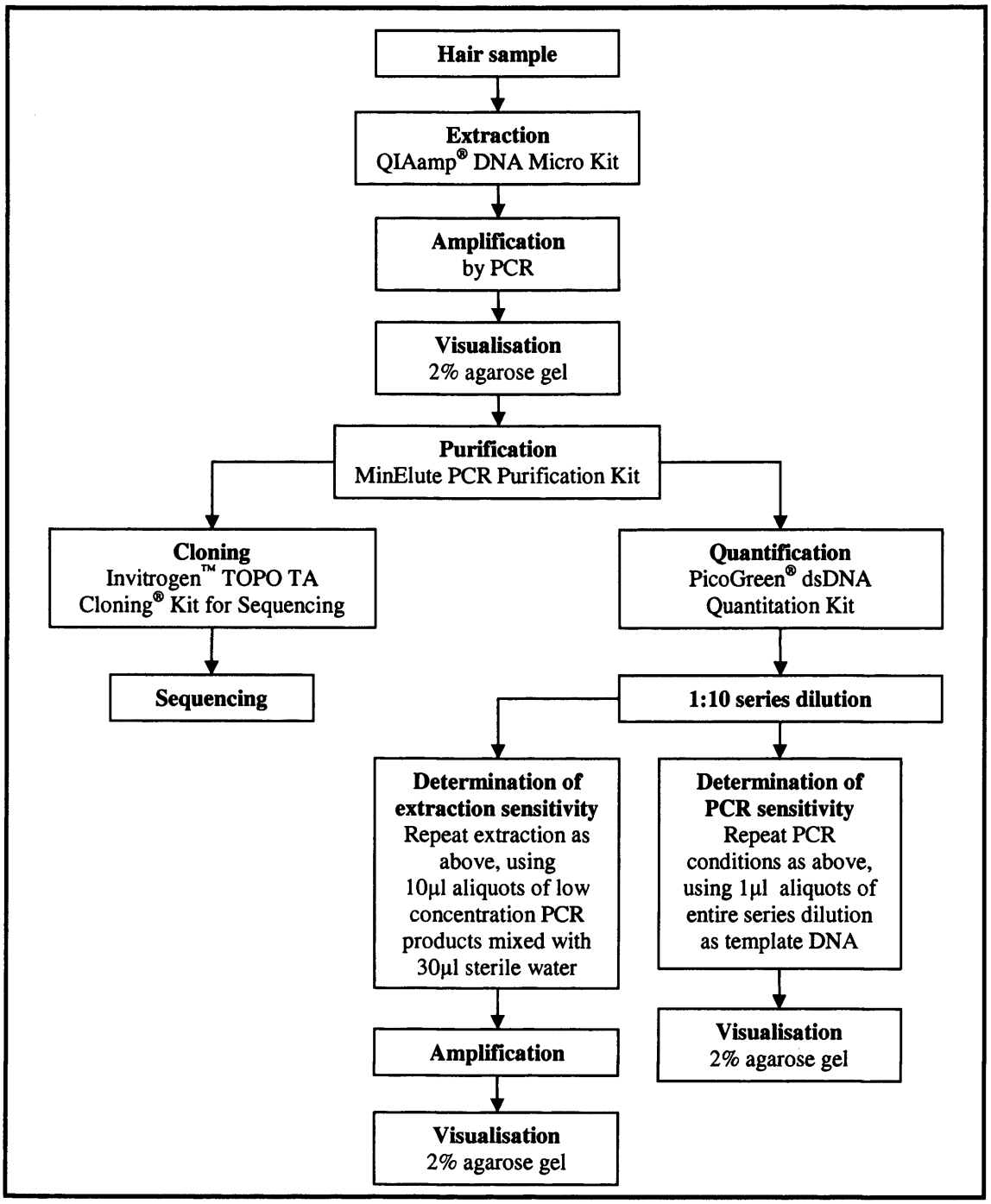


Figure 4. Flowchart outlining methods used in the mummy case study. Not all samples were used in each step as presented, for reasons outlined in text.

4.2.1. Selection of mummy samples

Hair samples were collected from eight cat (*Felis silvestris* Schreber 1775) mummies at the Natural History Museum, London. Accession records indicate that the mummies were excavated *c.*1900 by Sir William Flinders Petrie, possibly from Abydos, however the exact provenance and date of collection are unknown. The specimens used, their accession numbers, any relevant catalogue information, and the lab numbers issued for this study are outlined in Table 9.

Accession number	Catalogue information	Lab number
1979 5360		IA 1
1979 5398		IA 2
1979 5393		IA 3
1979 5387		IA 4
1979 5390		IA 5
1979 5372	2220 +/- 40 uncal, BM LAB No. 1547	IA 7
1979 5421	From Abydos	IA 8
1979 5420	wrappings 2110 +/- uncal (270 BC), calibrated date 380 BC, BM LAB No. 1548	IA 9

Table 9. List of ancient Egyptian cat mummy accession numbers, available catalogue information and lab numbers issued for this study.

All hair samples were collected from unwrapped areas of the mummies with clean forceps, and stored in sterile Eppendorf tubes.

Hair from the tail of a rabbit (*Oryctolagus cuniculus* (Linnaeus 1758)) mummified in 1999 was collected as above. The tail was donated by Dr. Salima Ikram from the American University of Cairo, from her collection of experimental animal mummies. The rabbit, referred to as Rabbit 4 (Fluffy) was eviscerated but not exsanguinated, washed with alcohol (Bold's gin served as a substitute for palm wine), filled with and buried in natron and exposed to the Cairo sun and air (for full details of the mummification process used, see Ikram 2005, 34-37). The natron was changed several times to facilitate desiccation over 27 days before the rabbit was wrapped in unbleached linen.

A recent cat hair was made available by supervisor, Dr. Ian Barnes, to confirm that the cat primers and PCR conditions were effective.

4.2.2. DNA extraction

All DNA extractions and set-up of subsequent PCR amplifications were conducted in a laboratory dedicated to ancient DNA analysis, and which was physically isolated from post-PCR amplification facilities. All equipment and surfaces were regularly decontaminated with a 5% sodium hypochlorite solution. Tubes and non-UV-sensitive solutions were irradiated with UV at 254 nm prior to use, and all glass and metal objects used were baked at >200°C for a minimum of 3 days prior to use.

To remove surface-contaminating DNA, all hair samples were initially washed in a 5% sodium hypochlorite solution for 10 seconds, followed by immersion in 95% ethanol for 10 seconds. In the first extraction of all 8 ancient cat and the one recent rabbit specimens, hair samples were not of a uniform length or size, due to the irregular nature of the ancient hairs which were quite brittle and broken, but several mid-shaft hair fragments were included in each extraction. A second extraction was undertaken of the modern rabbit mummy and the modern cat, which was not included in the first set of extractions to avoid contaminating the lab with recent cat DNA,

samples of which were restricted to a 1 cm mid-shaft segment of a single hair strand, each cut with a new disposable sterile scalpel blade.

DNA extractions were carried out using a QIAamp® DNA Micro Kit, following the "Protocol: Isolation of Genomic DNA from Forensic Case Work Samples" for "hair shafts without roots" (Qiagen 2003: 29-31) as follows:

1. Add 300 µl Buffer ATL, 20 µl Proteinase K, and 20 µl 1M DTT to a 1.5 ml micro-centrifuge tube. Add hair shaft sample. Close the lid and mix by pulse-vortexing for 10 s.

2. Place the tube in a heating block, and incubate at 56°C and vortex the tube for 10 s every 10 min.

3. Briefly centrifuge the tube to remove drops from the inside of the lid.

4. Add 300 µl Buffer AL, close the lid, and mix by pulse-vortexing for 10 s.

5. Place the tube in a heating block, incubate at 70°C and vortex the tube for 10 s every 3 min.

6. Centrifuge the tube at full speed (20,000 x *g*; 14,000 rpm) for 1 min.

7. Transfer the supernatant from step 6 to the QIAamp MinElute Column.

8. Close the lid, and centrifuge at 6000 x *g* (8000 rpm) for 1 min. Place the QIAampMinElute Column in a clean 2 ml collection tube, and discard the collection tube containing the flow-through.

9. Open the QIAamp MinElute Column and add 500 µl Buffer AW1. Close the lid and centrifuge at 6000 x *g* (8000 rpm) for 1 min. Place the QIAamp MinElute Column in a clean 2 ml collection tube, and discard the collection tube containing the flow-through.

10. Open the QIAamp MinElute Column and add 500 µl Buffer. Close the lid and centrifuge at 6000 x *g* (8000 rpm) for 1 min. Place the QIAamp MinElute Column in a clean 2 ml collection tube, and discard the collection tube containing the flow-through.

11. Centrifuge at full speed (20,000 x g; 14,000 rpm) for 3 min to dry the membrane completely.

12. Place the QIAamp MinElute Column in a clean 1.5 ml microcentrifuge tube and discard the collection tube containing the flow-through. Open the lid of the QIAamp MinElute Column and apply 20 µl Buffer AE to the centre of the membrane (in the second extraction, 50 µl Buffer AE was used).

13. Close the lid and incubate at room temperature (15–25°C) for 1 min. Centrifuge at full speed (20,000 x g; 14,000 rpm) for 1 min.

The final volume of eluate collected was approximately 18 µl in the first extraction, and approximately 45 µl in the second extraction.

A negative control containing no hair was taken through all phases of extraction and PCR.

4.2.3. PCR conditions and primers

One rabbit-specific primer pair and one cat-specific primer pair were designed, and used to generate PCR products from the cytochrome b region of the genome of both species (see Table 10 for primer names and sequences).

Primer name	Primer sequence (5'-3')	PCR product length (bp)
rabbit_cytbF	GAA TCC TCG TCG CAG ATC TTC T	133
rabbit_cytbR	CTT GCG AGG GGT ATG AGA ATA AG	
cat-cytbF	GCC AAC GGA GCT TCT ATA TTC	164
cat-cytbR	ATT TGG CCT CAT GGT AGG AC	

Table 10. PCR primers used for the rabbit and cat samples in this study.

Amplifications were carried out in a final reaction volume of 25 µl consisting of 1 µl of the DNA extract, 250 µM of dNTPs, 2mM $MgCl_2$, 1 mg/ml bovine serum albumin, 0.2 units of Platinum Taq HiFi (Invitrogen), and 1 µM of each primer. Cycling parameters were pre-incubation for 5 min at 92°C, followed by 45 cycles of 1 min at 92°C for denaturation, 1 min at 52°C for annealing, 1 min at 68°C for extension, and then a final incubation for 7 min at 68°C. Between one and three amplification blanks were included in each batch of PCRs.

PCR products were resolved by electrophoresis on 2% agarose gels. Failed amplifications were repeated to confirm initial results. Using series of 1/10 dilutions of template DNA of known concentration, it was determined that rabbit_cytbF/R were PCR-sensitive to between 6-59 molecules of the DNA template, and cat-cytbF/R were PCR-sensitive to 1-11 molecules of the DNA template, as visualised on 2% agarose gel.

4.2.4. Purification

To estimate the concentration of the PCR products in order to subsequently determine the efficiency of the PCR primers and conditions, sub-samples of PCR products resulting from the second extraction (recent cat and rabbit mummy) were first purified and then quantified using a

spectrophotometer.

Purification was carried out using a MinElute PCR Purification Kit, following the protocol as outlined in the "MinElute PCR Purification Kit Protocol using a microcentrifuge" (Qiagen 2001: 16) as follows:

1. Add 5 volumes of Buffer PB to 1 volume of the PCR reaction and mix (added 100 µl Buffer PB to 20 µl PCR product).

2. Place a MinElute column in a provided 2 ml collection tube.

3. To bind DNA, apply the sample to the MinElute column and centrifuge for 1 min.

4. Discard flow-through. Place the MinElute column back into the same tube.

5. To wash, add 750 µl Buffer PE to the MinElute column and centrifuge for 1 min.

6. Discard flow-through and place the MinElute column back in the same tube. Centrifuge the column for an additional 1 min at maximum speed.

7. Place the MinElute column in a clean 1.5 ml microcentrifuge tube.

8. To elute DNA, add 10 µl Buffer EB (10 mM Tris·Cl, pH 8.5) to the centre of the membrane, let the column stand for 1 min, and then centrifuge for 1 min.

Purified DNA (~10 µl) was then transferred to a 0.5ml tube and stored at 4°C.

4.2.5. Cloning and sequencing

Only the rabbit mummy PCR products were cloned and sequenced. PCR products were purified by adding 30 µl of 2/3 homemade MicroClean (2/3 HM-MC = 2 parts 40% PEG-8000, 1 M NaCl, 2 mM Tris-HCl (pH 7.5), 0.2 mM EDTA, 3.5 mM $MgCl_2$ and 1 part sterile water) to 10 µl of each amplification product, mixed well and incubated at room temperature for 10 minutes. Samples were then centrifuged at 4000 rpm for 45 minutes, and the liquid removed by slow centrifugation. To each sample, 150 µl of 70% ethanol was added, and centrifuged at 4000 rpm for 25 minutes, and the supernatant removed by slow centrifugation. Samples were then dried in a heating block at 65°C for 5 minutes. DNA was re-suspended in 80 µl of water, gently mixed and heated to 65°C for 5 minutes. The sequencing reaction was carried out in a final reaction volume of 15 µl, consisting of 5 µl Better Buffer (200mM Tris-HCl pH9, 5mM $MgCl_2$), 1 µl Termination Mix (ABI), 2.4 pM M13Forward/Reverse primers (5'-

GTAAAACGACGGCCAG-3'/5'-CAGGAAACAGCTATGAC-3') and 5.5 µl DNA. Cycling parameters were pre-incubation for 10 sec at 96°C, followed by 25 cycles of 5 sec at 50°C and 4 min at 60°C.

An Invitrogen™ TOPO TA Cloning® Kit for Sequencing was used to set up a 6 µl volume chemically competent *E. coli* cloning reaction consisting of 2 µl PCR product, 1 µl salt solution, 2 µl sterile water and 1 µl TOPO® vector. After gentle mixing and incubation for 5 minutes at room temperature, the reaction was placed on ice, and the One Shot® Chemical Transformation Protocol (Invitrogen 2004: 8) was followed.

Sixteen colonies were randomly selected for PCR and sequencing. A 10 µl volume PCR reaction was set up for each colony, consisting of 1 µl buffer, 200 µM dNTPs, 1 µM M13Forward/Reverse primers, 0.04 units *Taq* polymerase and 7.8 µl sterile water. Cycling parameters were pre-incubation for 5 min at 94°C, followed by 30 cycles of 1 min at 94°C for denaturation, 1 min at 50°C for annealing and 1 min at 72°C for extension, with a final incubation at 72°C for 10 min.

4.2.6. Quantification

To 10 µl of both the rabbit and modern cat purified PCR products, 40 µl of ultrapure water was added. The PicoGreen® dsDNA Quantitation Kit and a Perkin Elmer LS55 Luminescence Spectrometer were used.

1 µl of each sample was placed in a cuvette, and a solution made up to 1ml with 1xTE buffer. Another 1.0 ml of aqueous PicoGreen reagent was added to the sample, which was incubated for 2-5 minutes at room temperature, protected from light.

Curve calibration standards were mixed using the same buffer and PicoGreen reagent with PicoGreen dsDNA, following the protocol given in the Product Information dated 28-January-2003. Calibration standards of the following concentrations were used: blank, 1 ng/mL, 2.5 ng/mL, 10 ng/mL, 25 ng/mL, 100 ng/mL and 1 µg/mL. A calibration curve was plotted using Excel 2003.

4.2.7. Series dilution

A 1/10 series dilution was set up (adding 10 µl of purified PCR product to 90 µl ultrapure water) in order to have a range of known concentration PCR product solutions. These solutions were used to determine the sensitivity of both the extraction and PCR procedures.

4.2.8. Determination of extraction sensitivity

Low concentration samples of purified cat PCR products (1-114,000 copies) from the serial dilution were used to test the sensitivity of the extraction process. The cat serial dilution

samples were chosen, because the concentration range minimum was, theoretically, 1 copy of DNA, as opposed to 5 in the rabbit dilution series. Aliquots of 10 µl of the serial dilution solutions were mixed with 30 µl sterile water, and the resulting solutions were taken through the same extraction procedure as the original hair sample. One extraction blank was also run. Final elution was with 40 µl of AE buffer. A 1µl aliquot of the final elution solutions was used as template DNA in a PCR using the original conditions. A PCR blank was also run. The sensitivity of the extraction technique was determined by visualisation on a 2% agarose gel (if bands could not be visualised, the sample was deemed to have failed). This procedure was repeated once, because of a false positive in the first PCR blank. All subsequent PCR blanks were clean.

4.2.9. Determination of amplification sensitivity

To determine the sensitivity of the PCR conditions, 1 µl of 1/10 series dilution solutions was used as the template DNA in another PCR using the same conditions as before. The full range of the series dilutions of both the cat and the rabbit were used. The series dilution was continued beyond the theoretical absence of DNA (i.e. less than 1 copy of DNA present) four times (functioning as blanks), as well as two PCR blanks. Again, the sensitivity of the extraction technique was determined by visualisation on a 2% agarose gel (if bands could not be visualised, the sample was determined to have failed).

Chapter 5. Results

Data gathered from the screening test was analysed using qualitative, semi-quantitative, quantitative and statistical methods, to assess the effects of chemical treatments on DNA. A wide range of methods were used to check that results obtained were consistent using different means of data analysis. Qualitative assessments of damaged based on electropherograms were made, and a scoring system was devised to semi-quantitatively assess peak height ratio data in boxplots and scatterplots. Peak height ratio data were also used to calculate the percentage of DNA preservation for each treatment group compared to the control, and the Mann-Whitney test was used to compare the peak height ratio of each treatment group with the water control to further support the conclusions reached. Differences between repeated runs of samples were also explored using the Wilcoxon signed ranks test. Lists of chemicals safe and unsafe to use were compiled based on their effects on DNA as found in the screening test.

The effects of mummification in the short term using ancient Egyptian methods, as currently understood, are reported both in respect to the analysis of the collection material used and in light of the results of the screening test, as chemicals similar to those used in the mummification process (gin and natron) were also screened (ethanol and sodium carbonate). The sensitivity of the DNA extraction and PCR amplification methods used in the case study are also explored.

5.1. Screening test

The effects of the chemical treatments administered to short strands of DNA were assessed by visual inspection of electropherograms, and using scatterplot and boxplot diagrams. Scoring systems were created to semi-quantitatively assess the scatterplot and boxplot diagrams. The Mann-Whitney test was also carried out, which is a non-parametric test that compares two unpaired groups to test whether the two groups are from the same population (Field 2005). The Mann-Whitney test was used to compare all treatments against a water control, organic solvent-based treatments were also compared against the solvent alone, and heated treatments were compared against unheated treatments and heated water controls. Each sample was analysed twice, and to investigate the effect of conditions between sample runs, the Wilcoxon signed ranks test was used. The Wilcoxon signed ranks test is a non-parametric test used to compare the distribution of two paired groups, taking into account the magnitude of the difference between the two paired variables (Field 2005). Peak height ratios were also used to quantitatively determine the degree of DNA preservation relative to the control.

Test stock in water purified by reverse osmosis for 1 week was used as the control against which treated samples were compared. Although suspension in deionised water at room temperature would result in some damage to DNA, all treated samples were exposed to the same

volume of water at room temperature, so use of such a control negated any shared effects by controlling for these variables.

5.1.1. Electrophoresis observations

Upon visual inspection of the electropherograms, damage to the treated DNA could often be identified by a marked reduction in the peak height of the test sequences compared to the standard sequences. See Figure 5 for examples of a water control electropherogram compared to a mineral oil electropherogram showing peaks for damaged DNA.

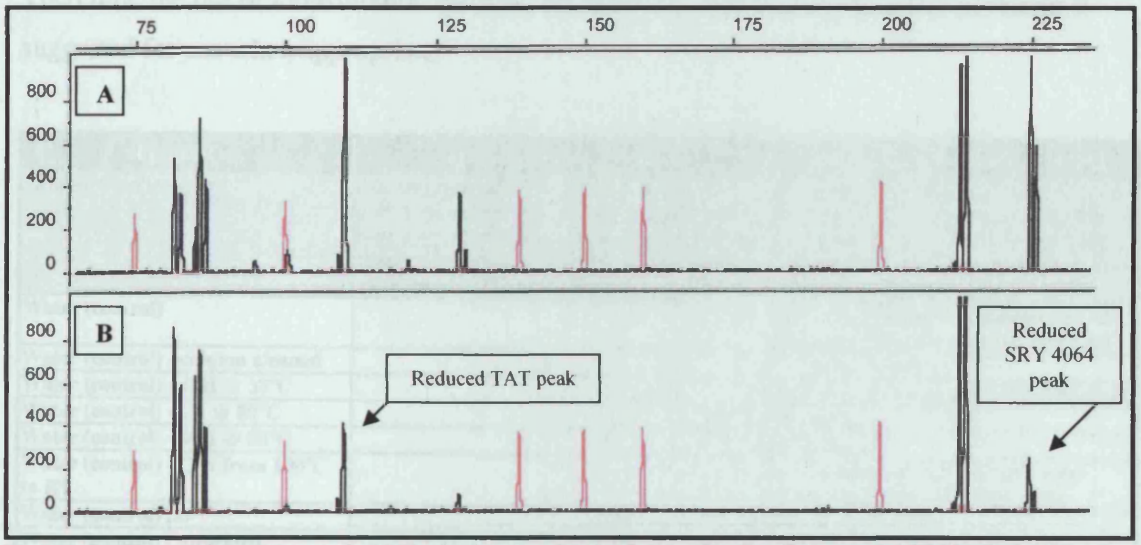


Figure 5. Electropherograms of: A) Typical water (control) sample after 7 days, and B) Typical mineral oil sample after 7 days. Note the reduction in TAT and SRY 4064 peak heights in the mineral oil electropherogram, indicative of damage.

Visual observations of sample changes during the experiment and inspection of electropherograms are presented in Table 11. Damage was documented if test peaks were notably reduced compared to standard peaks. Damage was also noted if size standard peaks were present, but test and standard stock peaks were absent, and the chemical used for the treatment was not prone to precipitation under the test conditions (e.g. sodium hydroxide). This is because the size standard was added to samples just prior to CE, after sample clean-up and DNA precipitation; if a treatment chemical did not co-precipitate with the DNA, it should have been removed during sample clean-up and therefore it would not have attacked the size standard, but the lack of test and standard stock peaks indicate that all DNA in the sample was destroyed by the chemical treatment. For some of the treatments tested and the chemical co-precipitated with the DNA (see Section 4.1.3), the presence of ions remaining in the sample interfered with the electrophoresis, resulting in no identifiable peaks (including the size standard) or other anomalies in the electropherograms. These samples (alum, detergent, EDTA, gum arabic, mercury (II) chloride, oxalic acid, sodium bicarbonate and sodium perborate) were subjected to additional clean-up measures, namely re-precipitation in chilled 70% ethanol ("re-ppt") or dialysis spin column purification ("column cleaned"), and the results of these re-

processed samples are also given. During the re-precipitation clean-up trial, it was observed that due to the added mass of chemical crystals, the DNA pellet was more likely to be lost in sample processing, which explains the tendency for these samples to yield size standard peaks only. This clean-up method did not substantially improve the results for any of the treated samples tested, therefore, this clean-up measure is not considered efficient. Purification using an ultrafiltration column was tested on alum, oxalic acid and sodium perborate treated samples, and although the alum and oxalic acid samples yielded size standard only, the sodium perborate sample that was column cleaned was the only sample to yield useable data for this treatment. Therefore, the use of ultrafiltration columns for resolving chemical precipitation problems is suggested for use when appropriate.

Treatment	Treatment observations	Electropherogram observations
(2)=repeated treatment	*No entry = no unusual observations noted during the experiment. ppt = precipitate; PPT expt = precipitation experiment, which was the testing of lower concentrations of chemicals to avoid co-precipitation with DNA.*	*Damaged=reduction in anticipated peak height or consistent splitting or lumping of sample peaks; Low peaks=peak heights <500, with many peak heights <300; Very low peaks=peak heights <100*
Water (control)		No visible damage (two samples had very low peaks and extra peaks, so excluded)
Water (control) - column cleaned		No visible damage
Water (control) - 48H @ 37°C		Possibly some damage in SRY 4064 peaks
Water (control) - 6H @ 80°C		Damaged
Water (control) - 40H @ 80°C		Damaged
Water (control) - O/N from 100°C to RT		Size standard only in 1 sample, some visible damage in SRY 4064 peaks
Water (control) (2)		No visible damage
Water (control) - unheated, unmixed (2)		No visible damage (one sample had no peaks, so excluded)
Water (control) - ether extracted (2)		No visible damage
Water (control) - 48H @ 37°C (2)		Possibly some damage in SRY 4064 peaks
Water (control) - 24H @ 80°C (2)		Damaged
Water (control) - 40H @ 80°C (2)		Damaged
Water (control) - O/N from 100°C to RT (2)		Damaged
Acetic acid		Some visible damage in SRY 4064 peaks
Acetone		No visible damage
1:1 acetone:IMS		No visible damage
Acrylic emulsion	Turned milky and remained milky after adding standard	Low peaks, but no visible damage
Alum	Treatment solution at upper limit of solubility when added; ppt formed during clean-up; in PPT expt, ppt formed w/ conc of 2.5% and higher in clean-up, and 2.5% re-dissolved in TE	No peaks
Alum (2)	Treatment solution at upper limit of solubility when added; ppt formed during clean-up; in PPT expt, ppt formed w/ conc of 2.5% and higher in clean-up, and 2.5% re-dissolved in TE	No peaks
Alum column cleaned		Size standard peaks only, no sample peaks = damaged
Alum re-ppt		Size standard peaks only, no sample peaks = damaged
Ammonium hydroxide	Upon adding treatment solution, immediately turned dark green; colorless within 4.5 hours; lighter blue than other samples after adding standard	Some low peaks, and some visible damage, in SRY 4064 peaks
Amyl acetate		No visible damage
Arsenic trioxide	Ppt formed during clean-up, but re-dissolved in TE	No visible damage
Benzene	Completely evaporated within 5 days	No visible damage
Carbon tetrachloride	Completely evaporated within 5 days	No visible damage
Cellulose nitrate	Excess treatment added (too viscous for pipette), mixed with DNA stock; white ppt and some trapped liquid remained after clean-up	Low peaks, but no visible damage

Treatment	Treatment observations	Electropherogram observations
(2)=repeated treatment	*No entry = no unusual observations noted during the experiment. ppt = precipitate; PPT expt = precipitation experiment, which was the testing of lower concentrations of chemicals to avoid co-precipitation with DNA.*	*Damaged=reduction in anticipated peak height or consistent splitting or lumping of sample peaks; Low peaks=peak heights <500, with many peak heights <300; Very low peaks=peak heights <100*
Chloroform	Mostly evaporated overnight	No visible damage
Detergent	Treatment solution solubility exceeded when added to sample; some bleaching effect immediately; colourless within 12 hours; light blue after adding standard; some gel/liquid remained after clean-up; in PPT expt, gel/ppt formed w/ conc of 2.5% and higher in clean-up, and (2.5% pellet lost, but) 5-10% re-dissolved in TE	Very low peaks with damage
Detergent (2)	Treatment solution solubility exceeded when added to sample; some bleaching effect immediately; colourless within 12 hours; light blue after adding standard; some gel/liquid remained after clean-up; in PPT expt, gel/ppt formed w/ conc of 2.5% and higher in clean-up, and (2.5% pellet lost, but) 5-10% re-dissolved in TE	No peaks or very low peaks with damage
Detergent re-ppt		Size standard peaks only, or untreated peaks only
Detergent - 40H @ 80°C	Treatment solution solubility exceeded when added to sample; sample mostly evaporated during experiment; lighter blue than other samples when re-suspended in TE; in PPT expt, gel/ppt formed w/ conc of 2.5% and higher in clean-up, and (2.5% pellet lost, but) 5-10% re-dissolved in TE	Low untreated peaks, no treated peaks
Detergent - 40H @ 80°C (2)	Treatment solution solubility exceeded when added to sample; sample totally evaporated by end; light blue after adding standard; in PPT expt, gel/ppt formed w/ conc of 2.5% and higher in clean-up , and (2.5% pellet lost, but) 5-10% re-dissolved in TE	Low untreated peaks, no treated peaks
EDTA	Treatment solution at upper limit of solubility when added to sample, in PPT expt, ppt formed w/ conc of 2.5% and higher in clean-up	No peaks, or very low peaks with damaged SRY 4064 peaks
EDTA (2)	Treatment solution at upper limit of solubility when added to sample, in PPT expt, ppt formed w/ conc of 2.5% and higher in clean-up	No peaks, or very low peaks with damaged SRY 4064 peaks
EDTA re-ppt		No peaks, or very low peaks with damage
Ethanol		No visible damage
1:1 ethanol:ether		Possibly some damage in SRY 4064 peaks
Ethyl acetate		No visible damage
Gasoline	Sample completely evaporated within 48 hours	Possibly some damage in SRY 4064 peaks
Gum arabic	Excess treatment added (too viscous for pipette); treatment did not mix with DNA stock; residue remained in tube after clean-up	Very low TAT peaks with SRY 4064 peaks missing
Gum arabic re-ppt		Very low peaks with damage
Hydrogen peroxide		Some visible damage, esp in SRY 4064 peaks
IMS		No visible damage
Kerosene		Possibly some damage in SRY 4064 peaks
Linseed oil	Solvent layer turned yellow/cloudy	Some visible damage in SRY 4064 peaks
Mercury (II) chloride	Ppt formed during clean-up, but re-dissolved in TE	Very low peaks with damage in SRY 4064 peaks or size standard only
Mercury (II) chloride (2)		Damaged
Mercury (II) chloride re-ppt		Size standard peaks only, no sample peaks = damaged
Methylmethacrylate/ethylacrylate	Solvent layer turned white/cloudy; residue remained after clean-up	No visible damage
Mineral oil		Damaged
Oxalic acid	Treatment solution solubility exceeded when added to sample; upon adding treatment, immediately turned green but clear (no ppt); yellow after 6 days; green after adding standard; in PPT expt, ppt formed w/ conc of 2.5% and higher in clean-up and 2.5-5% re-dissolved in TE	Size standard peaks only, no sample peaks = damaged

Treatment	Treatment observations	Electropherogram observations
(2)=repeated treatment	*No entry = no unusual observations noted during the experiment. ppt = precipitate; PPT expt = precipitation experiment, which was the testing of lower concentrations of chemicals to avoid co-precipitation with DNA.*	*Damaged=reduction in anticipated peak height or consistent splitting or lumping of sample peaks; Low peaks=peak heights <500, with many peak heights <300; Very low peaks=peak heights <100*
Oxalic acid (2)	Immediately turned green, still green after adding standard; Solubility exceeded in treatment stock when added to sample; in PPT expt, ppt formed in clean-up w/ conc of 2.5% and higher, 2.5-5% re-dissolved in TE;	No peaks
Oxalic acid column cleaned		Size standard peaks only, no sample peaks = damaged
Oxalic acid re-ppt		Size standard peaks only, no sample peaks = damaged
Pepsin		Low peaks with some visible damage, esp in SRY 4064 peaks
Pepsin (2)		Low peaks, damaged
Pepsin - 48H @ 37°C		Very low peaks with damaged or missing treated peaks
Pepsin - 48H @ 37°C (2)	Sample evaporated during experiment	Low peaks, damaged and often missing SRY 4064 peaks
Poly(vinyl) butyral resin	Residue remained after clean-up	Low peaks, but no visible damage
Potassium carbonate	Ppt formed during clean-up	Low peaks, but no visible damage
Potassium carbonate - 6H @ 80°C	Turned purple; sample partially evaporated after 4 hours	Low peaks with damage
PVAC	Excess treatment added (too viscous for pipette); blue/white ppt/residue remained after clean-up	Low peaks, but no visible damage
PVAC/PVAL	Turned milky and remained milky after adding standard; blue/white ppt/residue remained after clean-up	Low peaks, with possible damage in SRY 4064 peaks
Shellac	Turned milky and yellow, residue remained after clean-up	Low peaks, but no visible damage
Sodium bicarbonate	Treatment solution solubility exceeded when added to sample; blue/white ppt formed during clean-up; in PPT expt, ppt formed w/ conc of 2.5% and higher in clean-up and 2.5% re-dissolved in TE	No peaks, or very low peaks with some missing SRY 4064 peaks
Sodium bicarbonate (2)	Treatment solution solubility exceeded when added to sample; ppt formed during clean-up	No peaks, or very low peaks with some missing SRY 4064 peaks
Sodium bicarbonate re-ppt		Low peaks, but no visible damage
Sodium bicarbonate - 24H @ 80°C	Some evaporation during experiment; purple residue at bottom	Low peaks with some visible damage in SRY 4064 peaks
Sodium bicarbonate - 24H @ 80°C (2)	Sample mostly evaporated by end; gel-like residue formed during clean-up	Low peaks with some visible damage in SRY 4064 peaks
Sodium carbonate		Low peaks, but no visible damage
Sodium chloride		No visible damage
Sodium chloride - 24H @ 37°C	No control - treated with sodium chloride	No visible damage
Sodium hydroxide	Upon adding treatment solution, immediately turned green; purple within 4 hours; slightly purple after adding standard	Some visible damage in SRY 4064 peaks
Sodium hypochlorite	Upon adding treatment solution, immediately turned colourless, and remained colourless after adding standard	Size standard peaks only, no sample peaks = damaged
Sodium hypochlorite (2)	Upon adding treatment solution, immediately turned colourless, and remained colourless after adding standard	Size standard peaks only, no sample peaks = damaged
Sodium hypochlorite re-ppt		Size standard peaks only, no sample peaks = damaged
Sodium perborate	Treatment solution solubility exceeded when added to sample; within 10 minutes of adding treatment, sample turned from light blue to colourless; ppt formed during clean-up; in PPT expt, ppt formed w/ conc of 2.5% and higher in clean-up, and 2.5%-10% re-dissolved in TE	No peaks
Sodium perborate (2)	Some bleaching effect immediately, colourless by 12 hours, cloudy but colourless when isopropanol added; solubility exceeded in treatment stock when added to sample; in PPT expt, ppt formed in clean-up w/ conc of 2.5% and higher, 2.5%-10% re-dissolved in TE;	No peaks
Sodium perborate - O/N from 100°C to RT	Upon adding treatment solution, turned colourless	No peaks
Sodium perborate - O/N from 100°C to RT (2)	Some evaporation during experiment; turned light blue after adding standard; turned colourless/cloudy during clean-up	No peaks

Treatment	Treatment observations	Electropherogram observations
(2)=repeated treatment	*No entry = no unusual observations noted during the experiment. ppt = precipitate; PPT expt = precipitation experiment, which was the testing of lower concentrations of chemicals to avoid co-precipitation with DNA.*	*Damaged=reduction in anticipated peak height or consistent splitting or lumping of sample peaks; Low peaks=peak heights <500, with many peak heights <300; Very low peaks=peak heights <100*
Sodium perborate re-ppt		Size standard peaks only, no sample peaks (except 1 sample with very low peaks)
Sodium perborate column cleaned		Low peaks, but no visible damage
Sodium sulfide	Upon adding treatment solution, sample turned slightly green	Damaged
Sodium sulfide - 6H @ 80°C	Upon adding treatment solution, sample turned purple; partially evaporated after 4 hours	Damaged
Toluene		No visible damage
Trichloroethylene		No visible damage
Turpentine		Damaged
White spirit	Sample mostly evaporated within 5 days, and completely evaporated after 7 days	Some low peaks, but no visible damage
Xylene	Sample mostly evaporated overnight	No visible damage

Table 11. Summary table of experimental observations and electropherogram observations. "H" = hours, "O/N" = overnight, "RT" = room temperature.

All samples were included in the semi-quantitative and quantitative data analysis with the exception of two water controls and those treatments that resulted in no test or standard stock peaks. Two water control samples appeared to be extreme outliers in the initial data analysis. Upon re-inspection of the electropherograms, anomalies in the appearance of the test and standard stock peaks led to the decision to exclude those two samples from the data analysis. Either test and/or standard peaks were not produced for a few treatment samples, namely, alum, mercury (II) chloride re-ppt, oxalic acid, sodium hypochlorite and sodium perborate (prior to additional clean-up) therefore, their effects will be considered here.

Sodium hypochlorite sample electropherograms contained size standard only, suggesting that both the test and standard DNA was lost. As sodium hypochlorite did not chemically precipitate, it is unlikely that the DNA pellet was lost in all 4 replicate test samples twice (sodium hypochlorite was one of the treatments repeated a second time). It is therefore thought that sodium hypochlorite destroyed all test and standard DNA, as it is routinely used for that purpose in removing contaminant DNA from the surface of sample material (Kemp and Smith 2005).

The lack of evidence of DNA in the re-precipitated mercury (II) chloride sample is most likely due to treatment chemical precipitation and DNA pellet loss, as described above. As some data were obtained from the original mercury (II) chloride samples, the results of that treatment prior to additional clean-up are discussed below.

The lack of data from the sodium perborate samples, with the exception of the column cleaned sample, is considered a result of chemical precipitation ionically interfering with electrophoresis. The data gathered from the column cleaned sample are used to assess the effect of this treatment below.

The effects of alum and oxalic acid are less clear. Previous studies have reported the effects of "alum" on DNA analyses, but because the term "alum" has been used to refer to different compounds, it is difficult to summarise the published accounts succinctly (it should be noted that this problem is not exclusive to alum, see section 6.2). Williams (1999) published evidence of damage to DNA (as well as PCR inhibition) resulting from an experimentally administered treatment with "alum", which was aluminium ammonium sulfate ($AlNH_4(SO_4)_2$). Hall et al. (1992) reported that an additional water washing protocol was required for successful enzymatic digestion of tissue thought to have been treated with "alum" identified as potassium aluminium sulfate dodecahydrate ($KAl(SO_4)_2 \cdot 12H_2O$), based on energy dispersive x-ray microanalysis and scanning electron microscopy analysis of contaminants removed from historically prepared tissue samples. In this study, the alum used was potassium aluminium sulfate dodecahydrate, but enzymatic digestion methods were not used.

That the alum and alum (2) sample electropherograms showed no peaks at all, suggests ionic interference from chemical precipitate. The presence of size standard peaks, but neither test nor standard peaks in both the re-precipitated and column cleaned samples suggests DNA loss. What is unclear is whether the ionic interference in the original samples is masking total DNA loss.

Oxalic acid samples in the first instance provided electropherograms with size standard only, as did re-precipitated and column cleaned samples, suggesting total loss of DNA. This is reasonable, as oxalic acid is a relatively strong acid, and DNA is susceptible to acid catalysed damage (Lindahl 1993). Repeated oxalic acid samples produced electropherograms with no peaks at all, suggesting ionic interference was a stronger influence for these samples, possibly due to the oxalic acid stock solution becoming more concentrated due to evaporation. This implies that it is possible for ionic interference to mask total DNA loss, and this effect may be what was observed in the alum and alum (2) samples above.

5.1.2. Scatterplot observations

Scatterplots were created using Excel 2003 to provide a preliminary assessment of treatment effects and consistency of effect across replicate treatment samples (see Figure 6 to Figure 29). In each scatterplot, the TY ratio was plotted on the x-axis, and the SM ratio on the y-axis. For the scatterplots, only the A and B peak ratios were used, as only A and B peaks were available for the SRY 4064 and M9 sequences. The TAT A and B peaks were used in the scatterplots, because the left-most peaks of the TAT sequence were consistently intact and readily identifiable, whereas the right-most peaks were occasionally split and would therefore yield less usable data. Cases where the standard was detectable, but the test DNA was not detectable (and therefore damaged) are represented as a zero value. These are shown in the scatterplots only,

but due to clustering around the zero value, not all points may be visible for each treatment sample. Each sample is represented as an individual point, and the water control data is additionally depicted with a single point at the mean, with x- and y- error bars extended to indicate standard deviation values.

For illustration purposes, treatments are presented in different scatterplots to highlight various associations. Unheated treatments were divided into water soluble treatments and organic solvent-based treatments. Heated treatments were plotted with their unheated equivalents and heated water controls. Heated water controls were also plotted separately to illustrate the effects of heating alone (as more heated water controls were tested using the second DNA stock, only these data are presented). Samples subjected to additional clean-up (re-precipitation or column cleaning) were plotted with their pre-additional-clean-up samples.

A scoring system was devised to rate the relative effect of each treatment based on the placement of replicate samples compared to the water (control) mean and standard deviation. Scores were allocated to the TY and SM ratios separately for each treatment group, for both replicate sample runs. Scoring criteria were as follows:
2 = all treated samples $\geq$ water control standard deviation
1 = all treated samples $\geq$ water control mean
0 = treated samples above and below water control mean
-1 = all treated samples $\leq$ water control mean
-2 = all treated samples $\leq$ water control standard deviation
Ranked scores are summarised in Table 12.

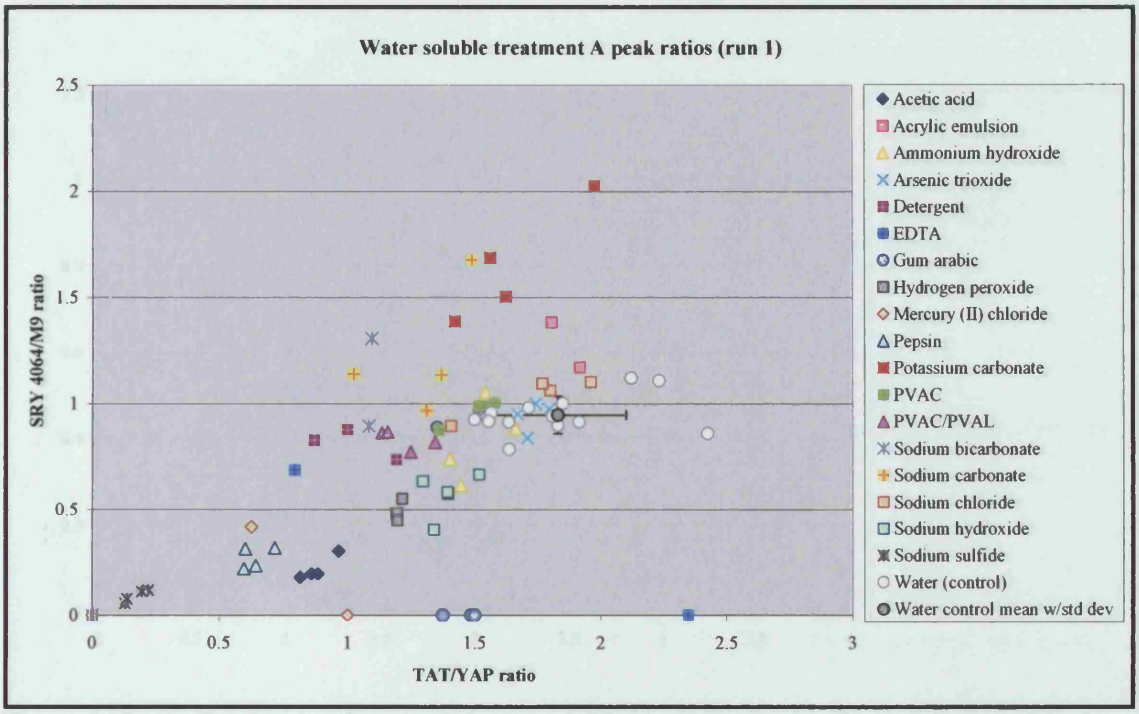


Figure 6. Scatterplot showing the unheated water soluble treatment A peak ratios (run 1), "w/std dev" = with standard deviation. Effects of treatments are summarised in Table 12.

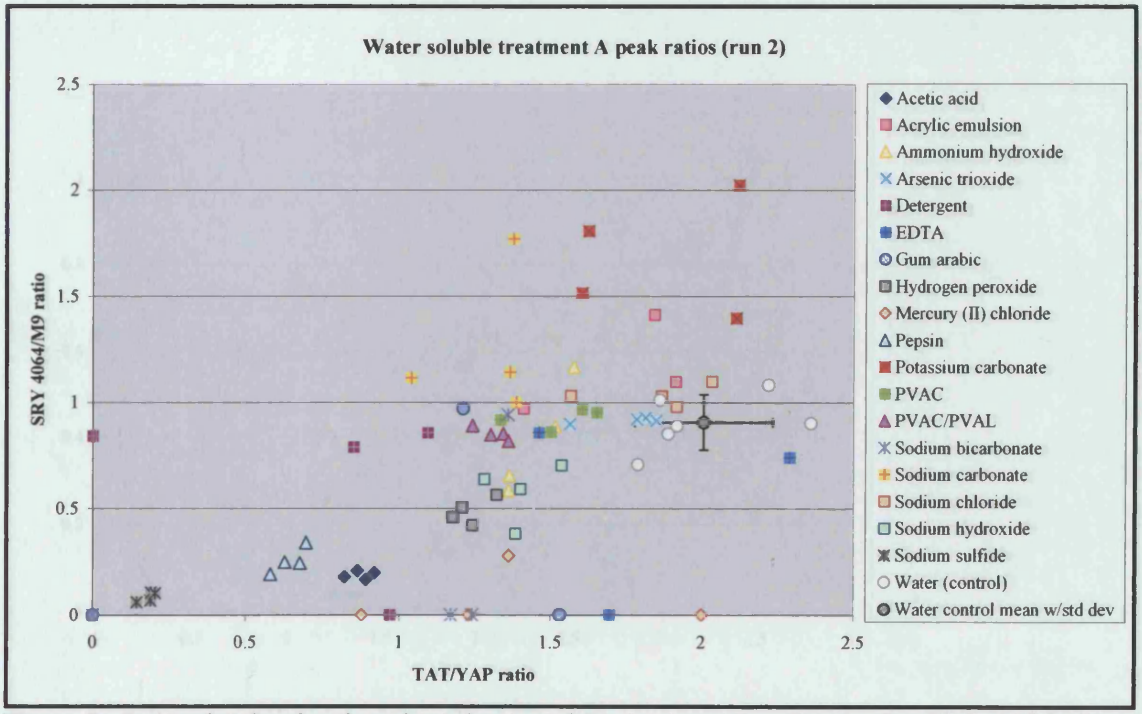


Figure 7. Scatterplot showing the unheated water soluble treatment A peak ratios (run 2), "w/std dev" = with standard deviation. Effects of treatments are summarised in Table 12.

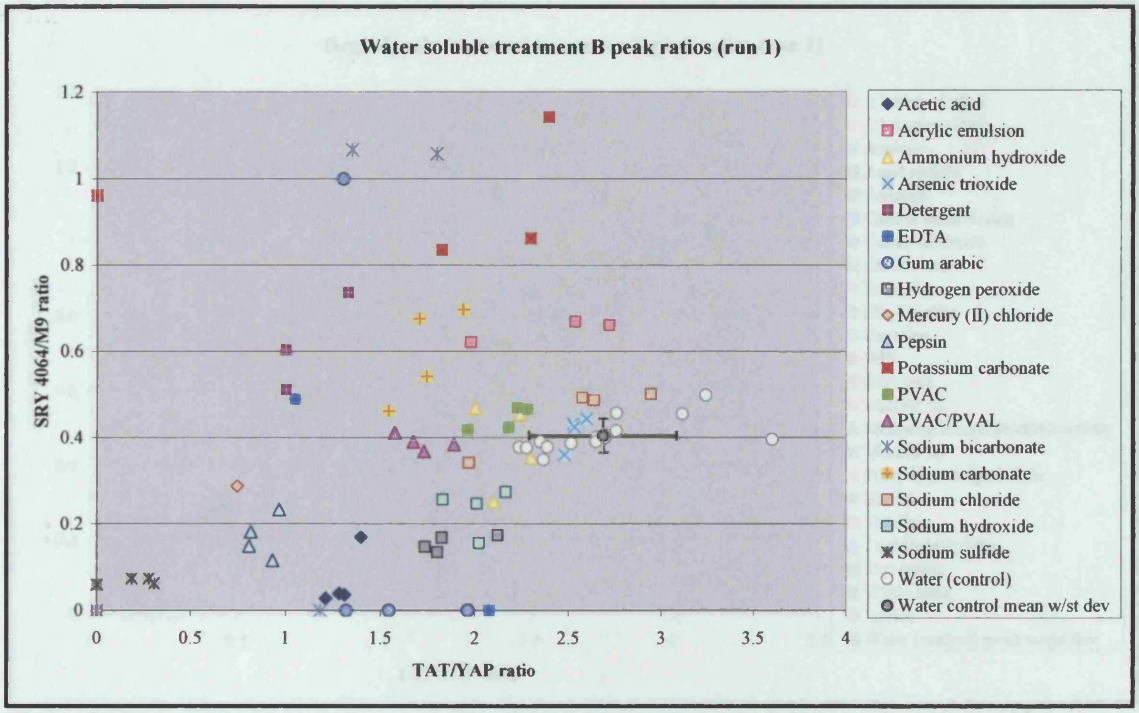


Figure 8. Scatterplot showing the unheated water soluble treatment B peak ratios (run 1), "w/std dev" = with standard deviation. Effects of treatments are summarised in Table 12.

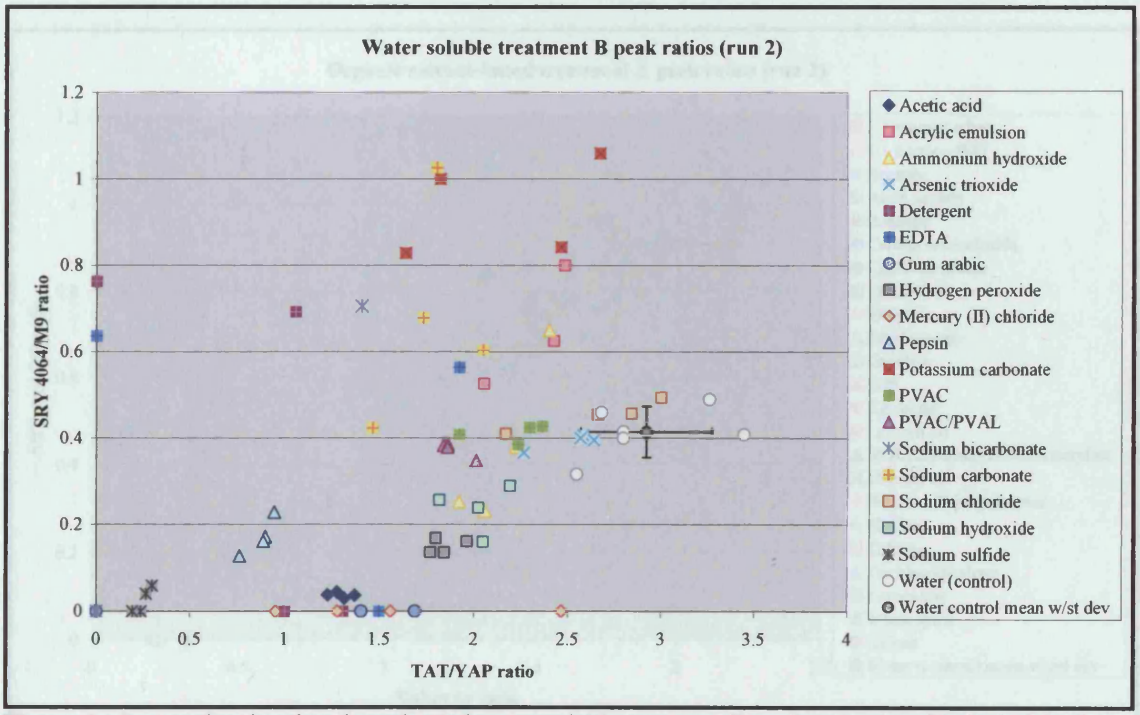


Figure 9. Scatterplot showing the unheated water soluble treatment B peak ratios (run 2), "w/std dev" = with standard deviation. Effects of treatments are summarised in Table 12.

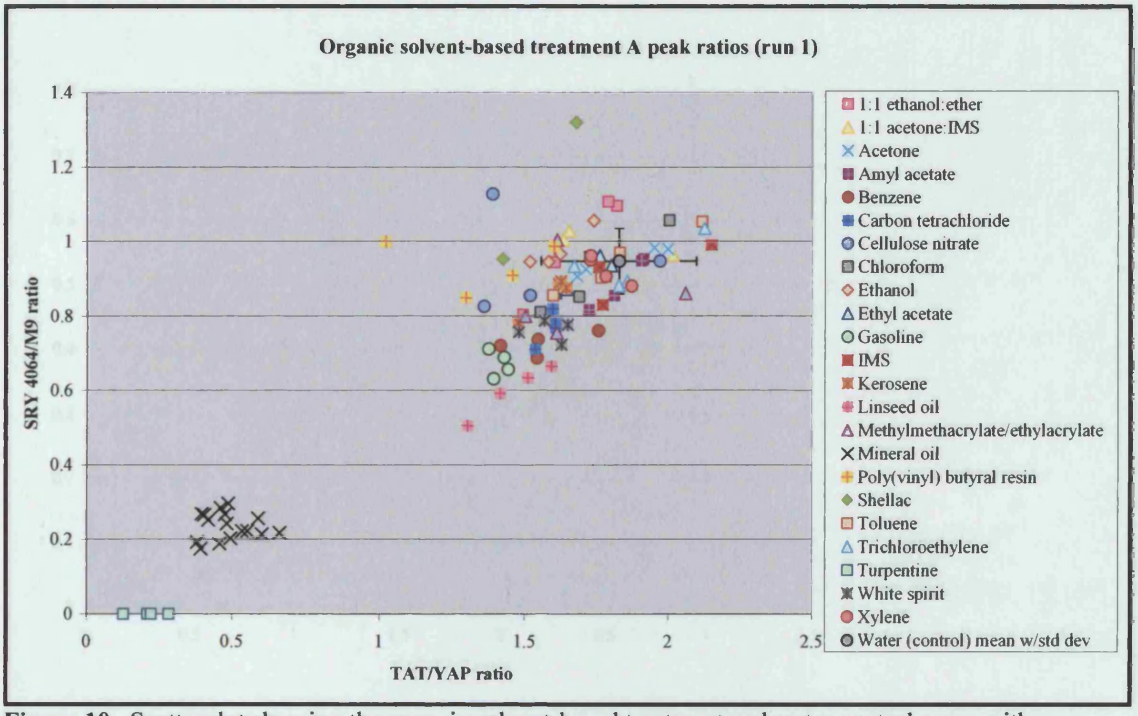


Figure 10. Scatterplot showing the organic solvent-based treatment and water control mean with standard deviation A peak ratios (run 1), "w/std dev" = with standard deviation. Effects are summarised in Table 12.

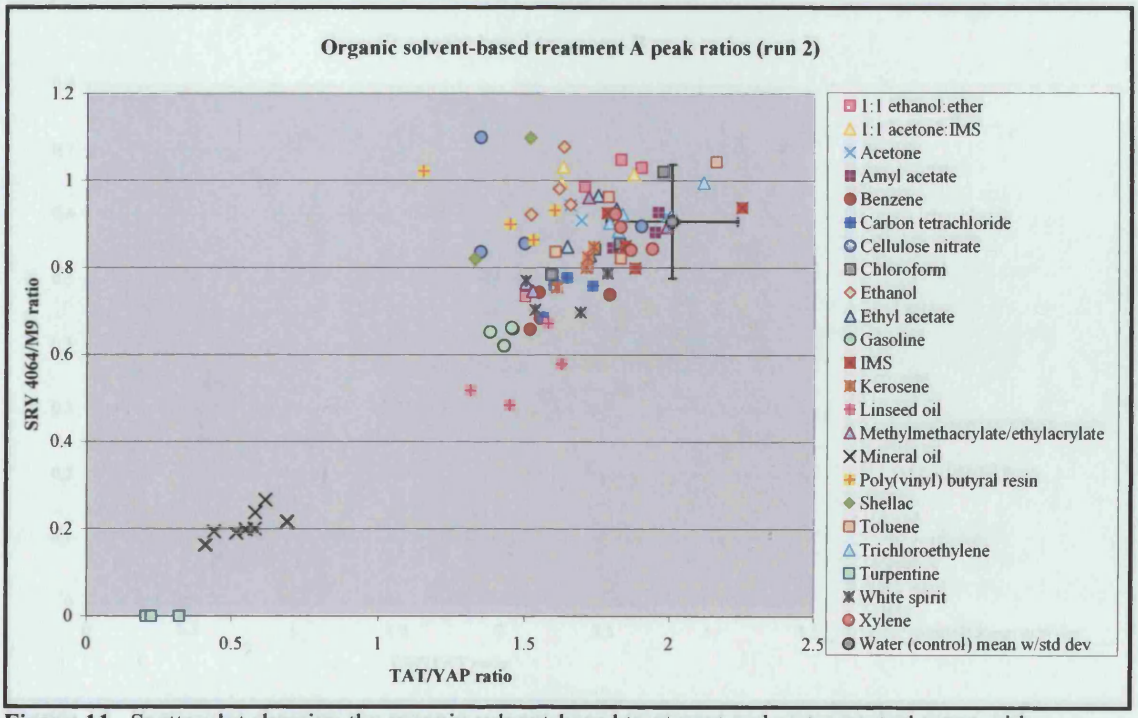


Figure 11. Scatterplot showing the organic solvent-based treatment and water control mean with standard deviation A peak ratios (run 2), "w/std dev" = with standard deviation. Effects are summarised in Table 12.

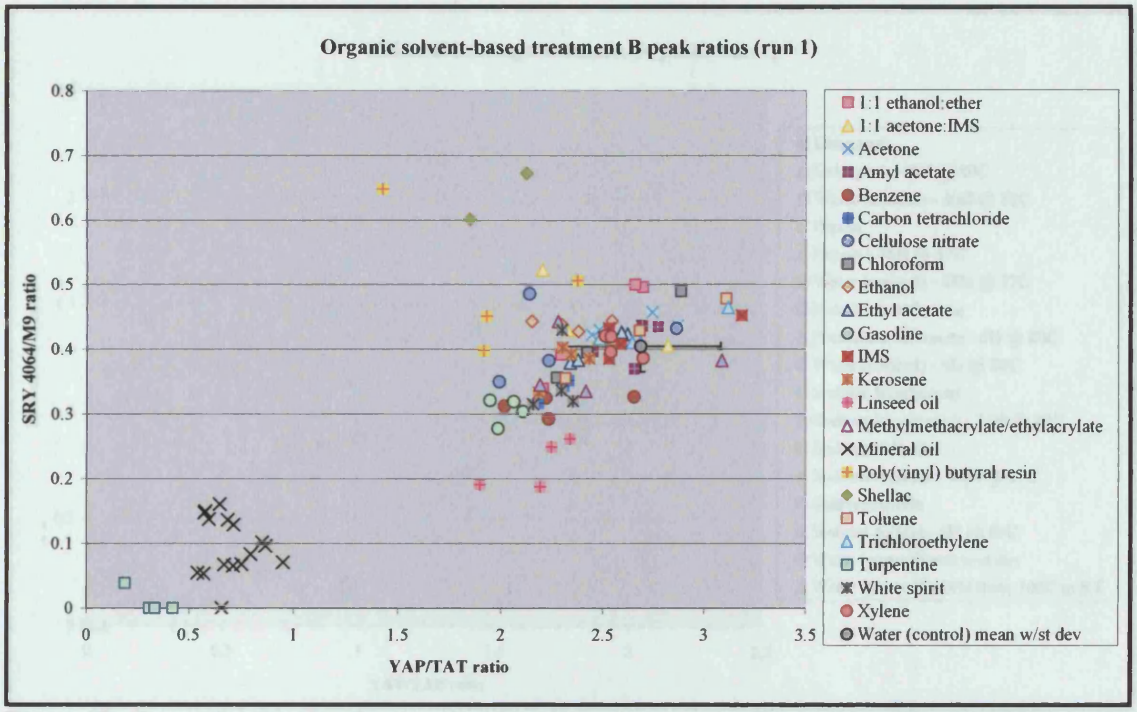


Figure 12. Scatterplot showing the organic solvent-based treatment and water control mean with standard deviation B peak ratios (run 1), "w/std dev" = with standard deviation. Effects are summarised in Table 12.

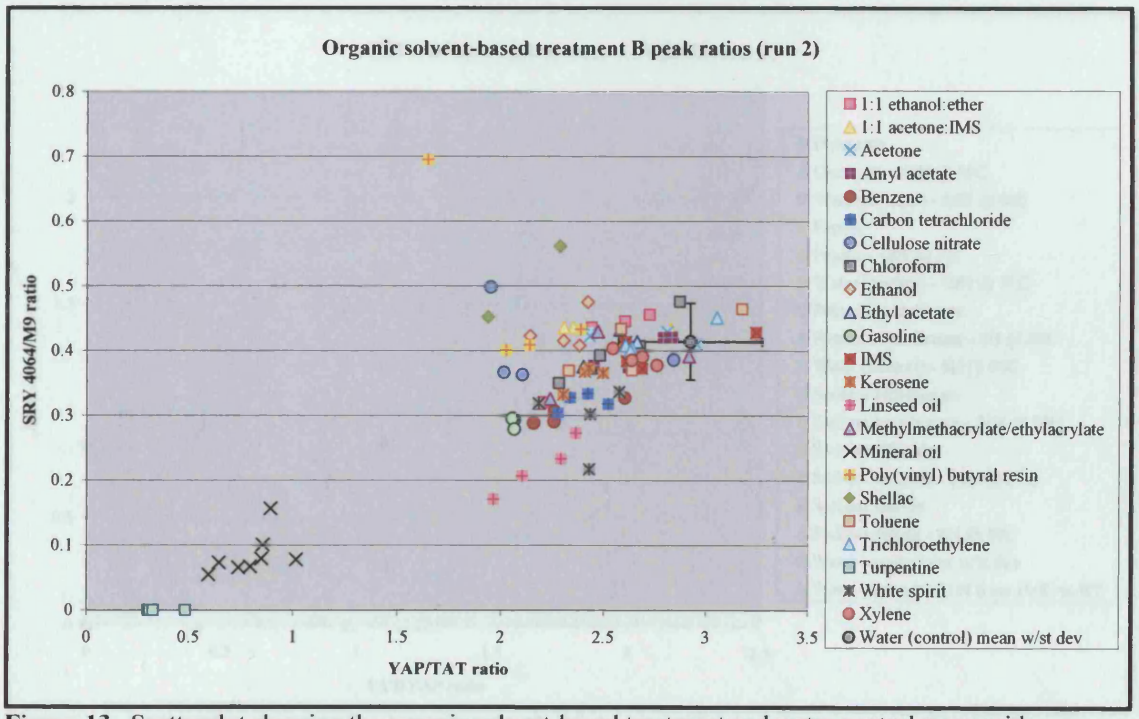


Figure 13. Scatterplot showing the organic solvent-based treatment and water control mean with standard deviation B peak ratios (run 2), "w/std dev" = with standard deviation. Effects are summarised in Table 12.

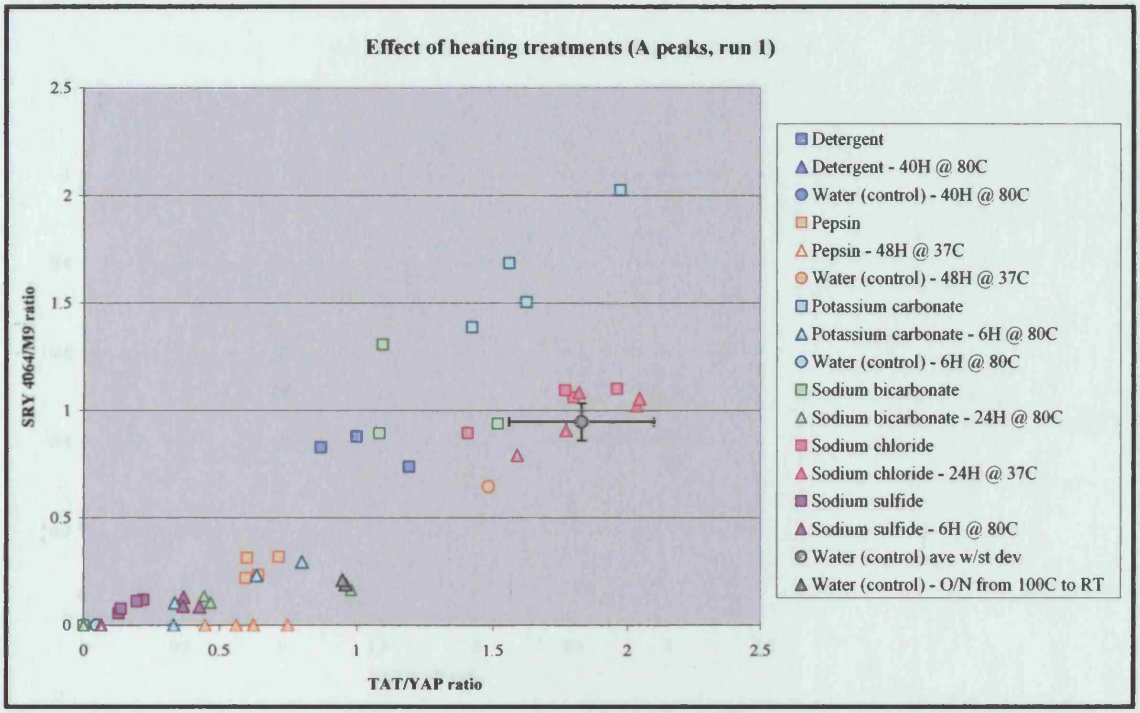


Figure 14. Scatterplot showing the heated and unheated water soluble treatment A peak ratios (run 1), "ave w/std dev" = average with standard deviation, "H" = hours, "O/N" = overnight, "RT" = room temperature. Effects are summarised in Table 12.

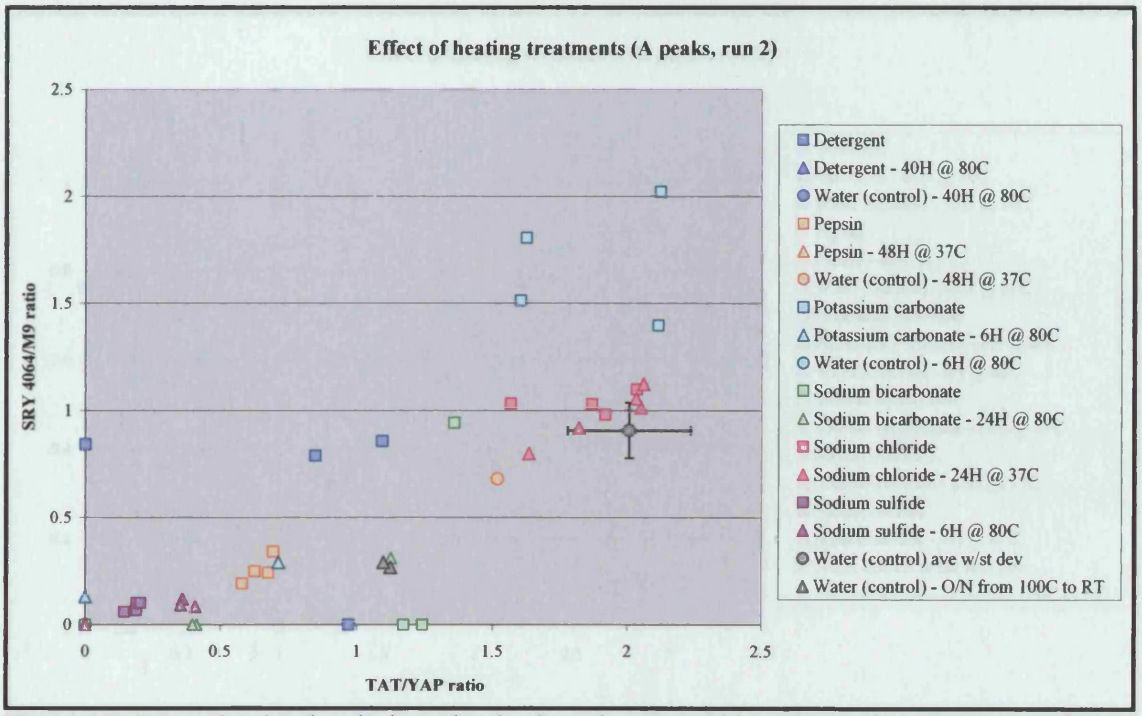


Figure 15. Scatterplot showing the heated and unheated water soluble treatment A peak ratios (run 2), "ave w/std dev" = average with standard deviation, "H" = hours, "O/N" = overnight, "RT" = room temperature. Effects are summarised in Table 12.

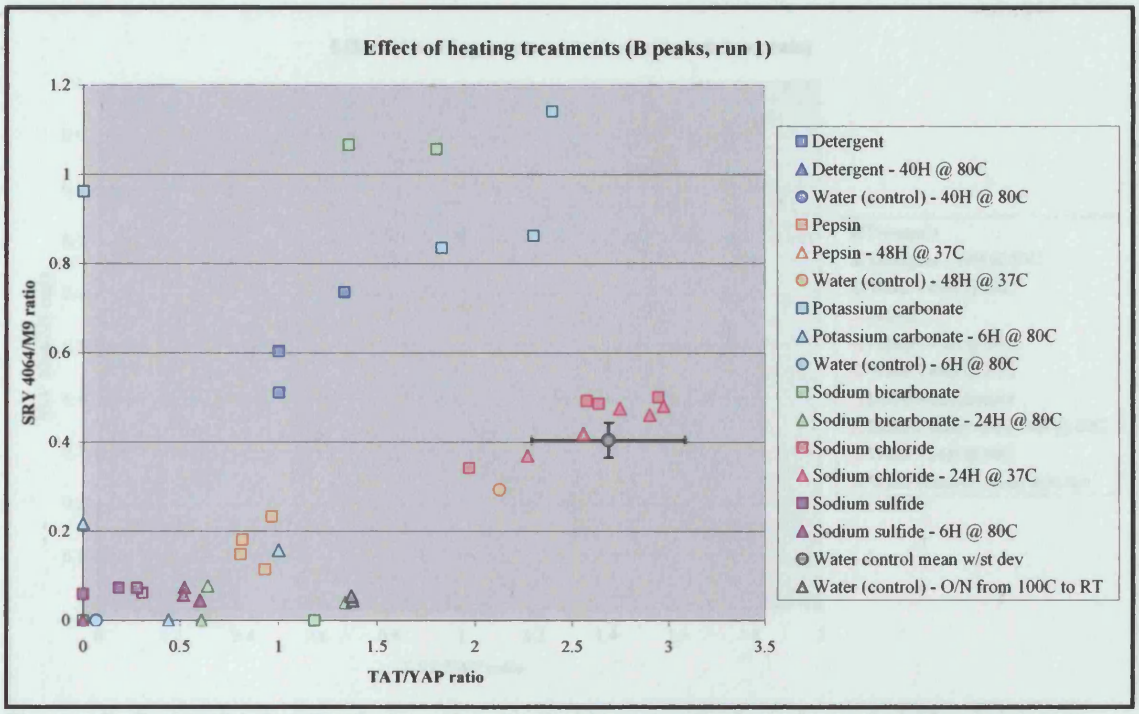


Figure 16. Scatterplot showing the heated and unheated water soluble treatment B peak ratios (run 1), "w/std dev" = with standard deviation, "H" = hours, "O/N" = overnight, "RT" = room temperature. Effects are summarised in Table 12.

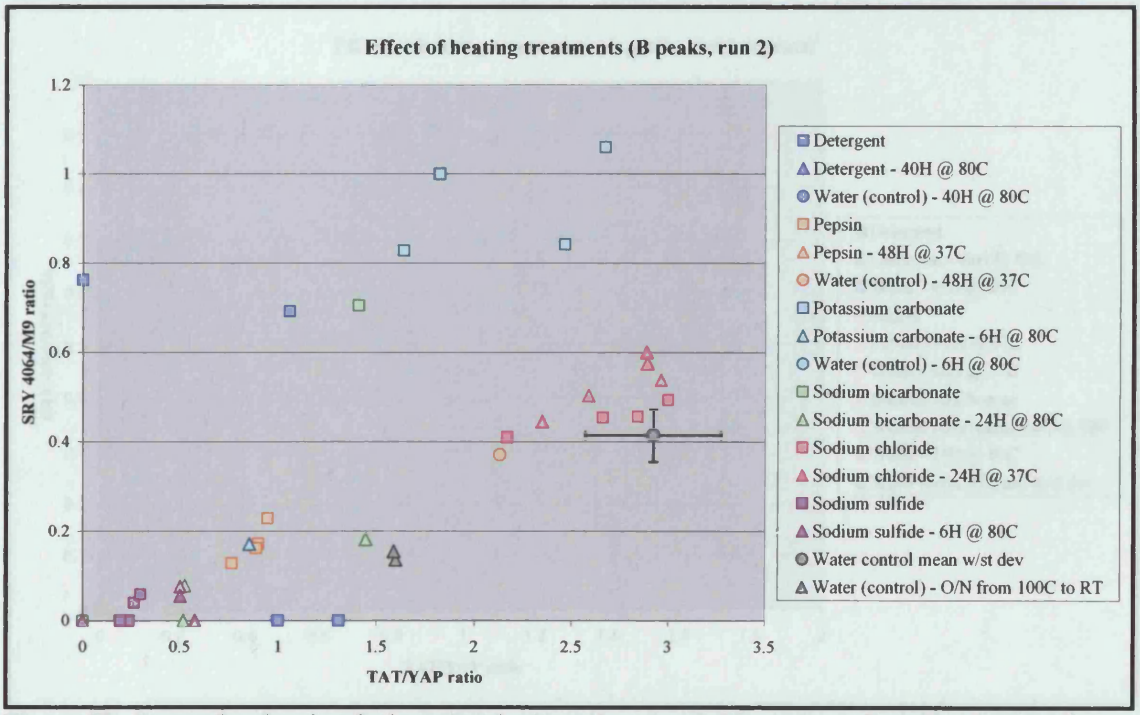


Figure 17. Scatterplot showing the heated and unheated water soluble treatment B peak ratios (run 2), "w/std dev" = with standard deviation, "H" = hours, "O/N" = overnight, "RT" = room temperature. Effects are summarised in Table 12.

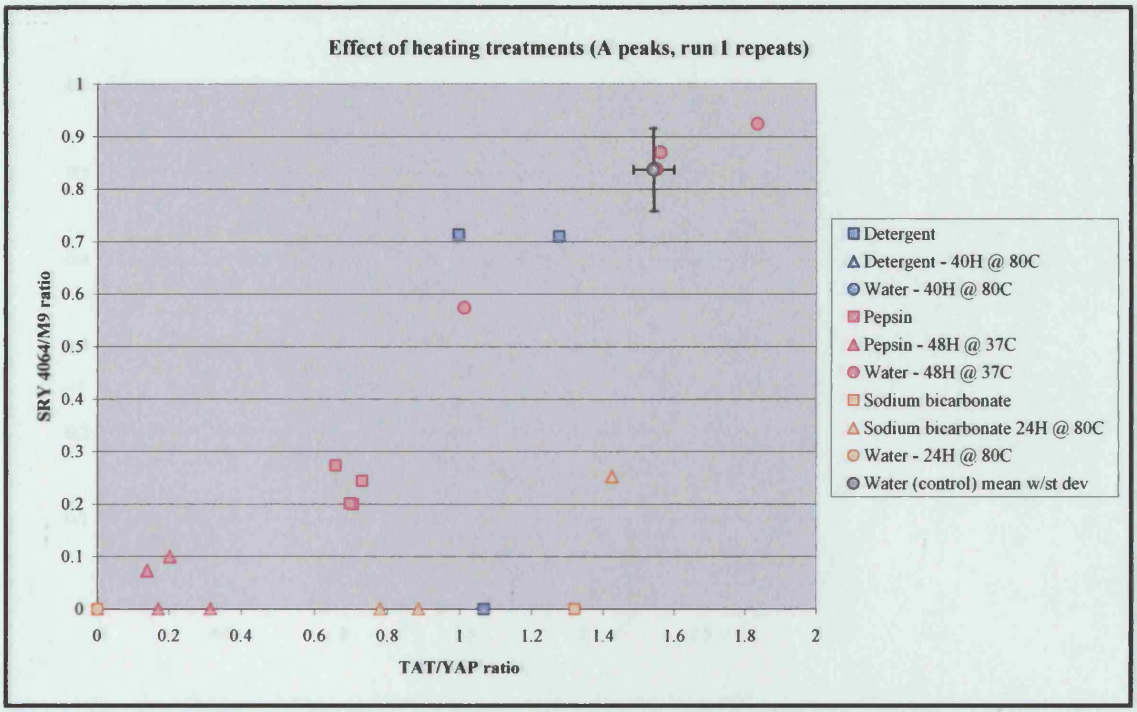


Figure 18. Scatterplot showing the repeated samples' heated and unheated water soluble treatment A peak ratios (run 1), "w/std dev" = with standard deviation, "H" = hours, "O/N" = overnight, "RT" = room temperature. Effects are summarised in Table 12.

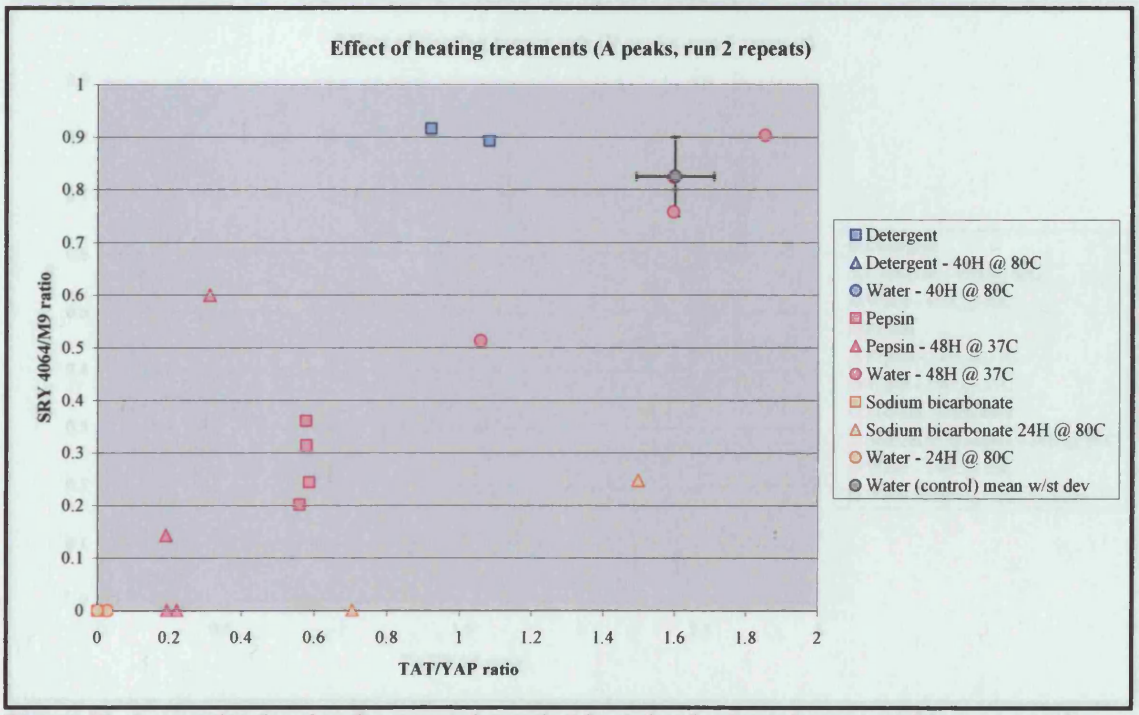


Figure 19. Scatterplot showing the repeated samples' heated and unheated water soluble treatment A peak ratios (run 2), "w/std dev" = with standard deviation, "H" = hours. Effects are summarised in Table 12.

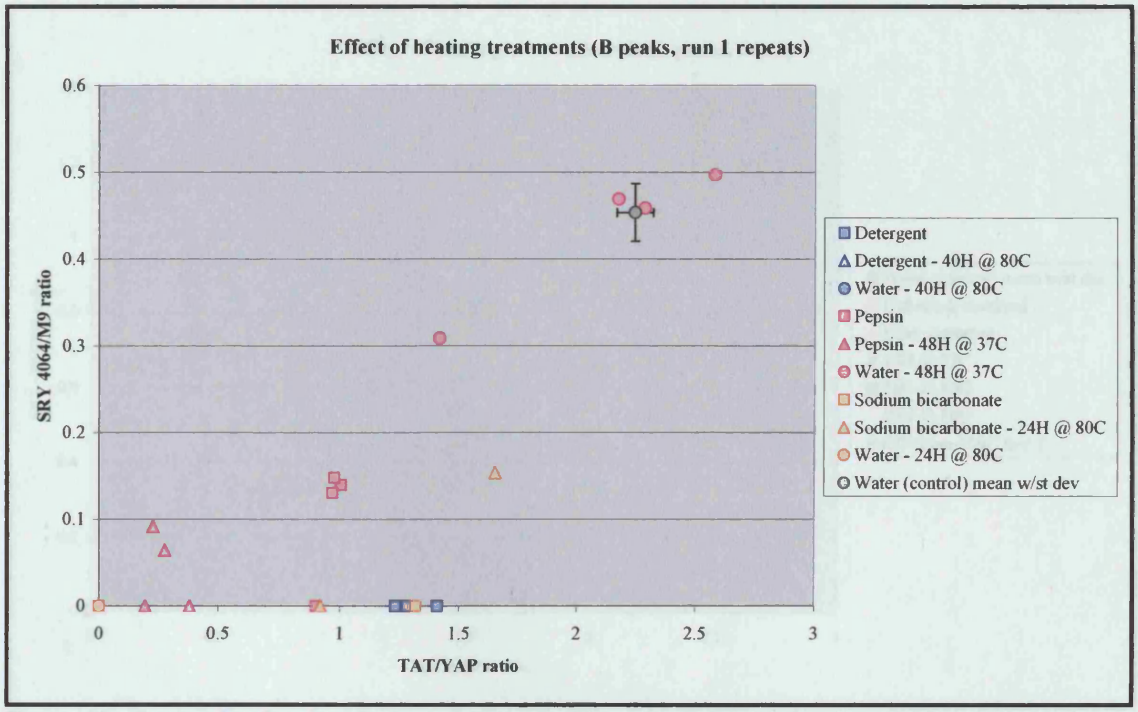


Figure 20. Scatterplot showing the repeated samples' heated and unheated water soluble treatment B peak ratios (run 1), "w/std dev" = with standard deviation, "H" = hours. Effects are summarised in Table 12.

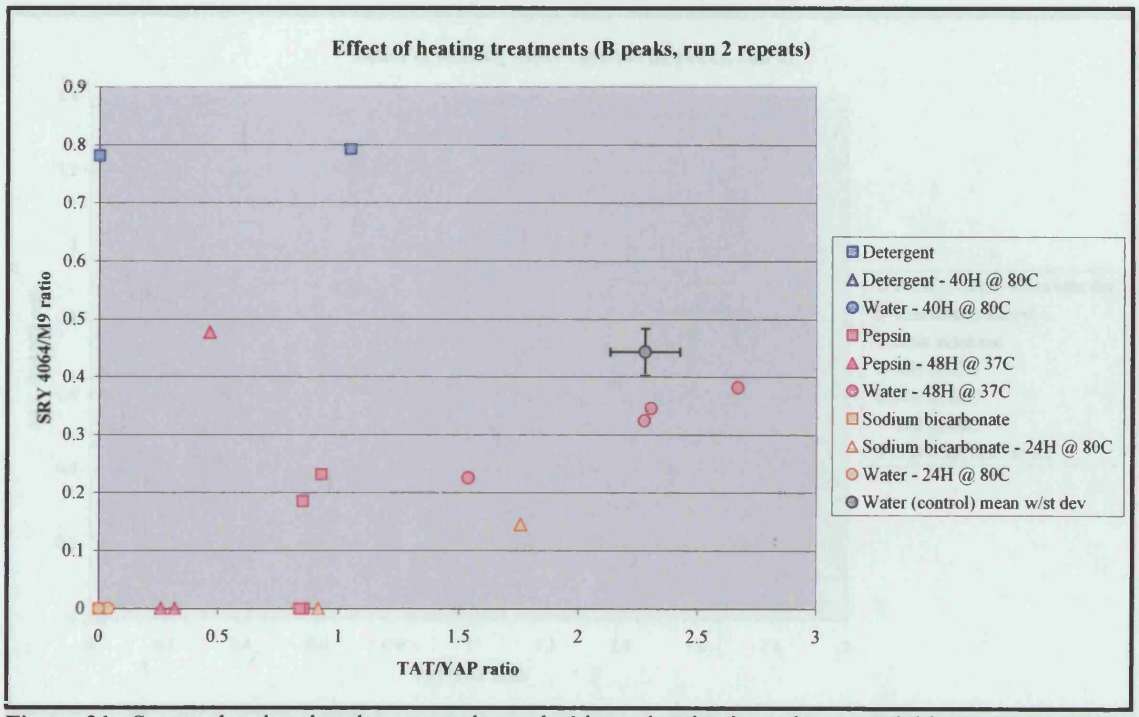


Figure 21. Scatterplot showing the repeated samples' heated and unheated water soluble treatment B peak ratios (run 2), "w/std dev" = with standard deviation, "H" = hours. Effects are summarised in Table 12.

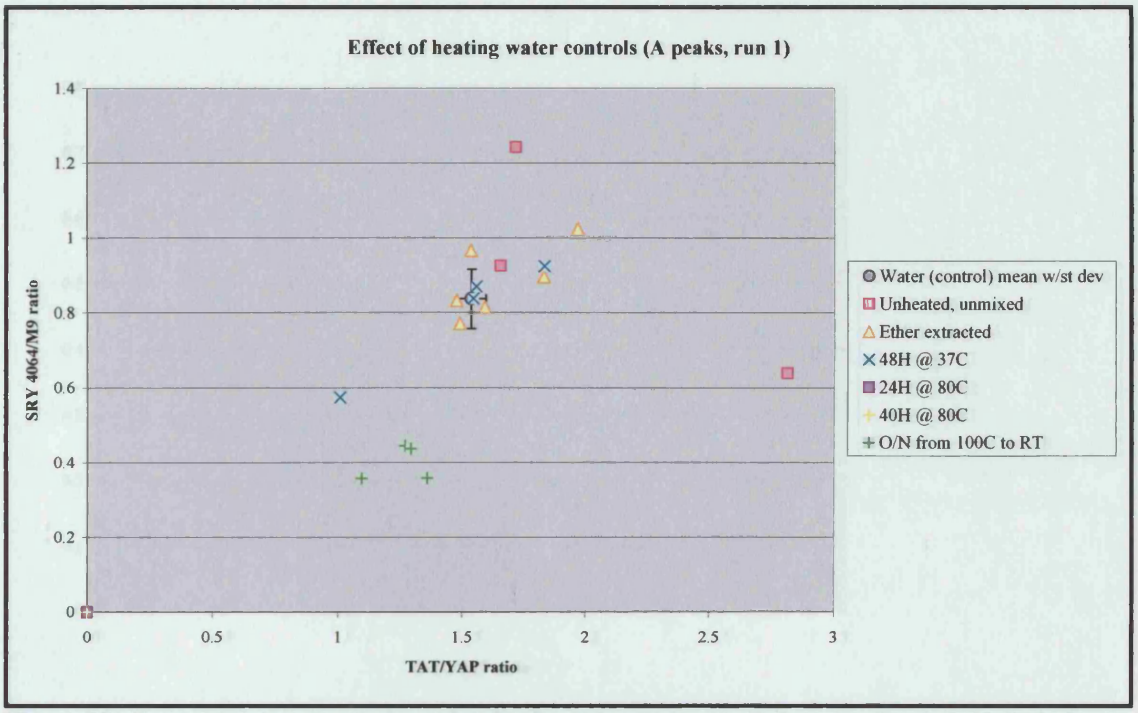


Figure 22. Scatterplot showing the heated and unheated water (control) A peak ratios (run 1), "w/std dev" = with standard deviation, "H" = hours, "O/N" = overnight, "RT" = room temperature. Effects are summarised in Table 12.

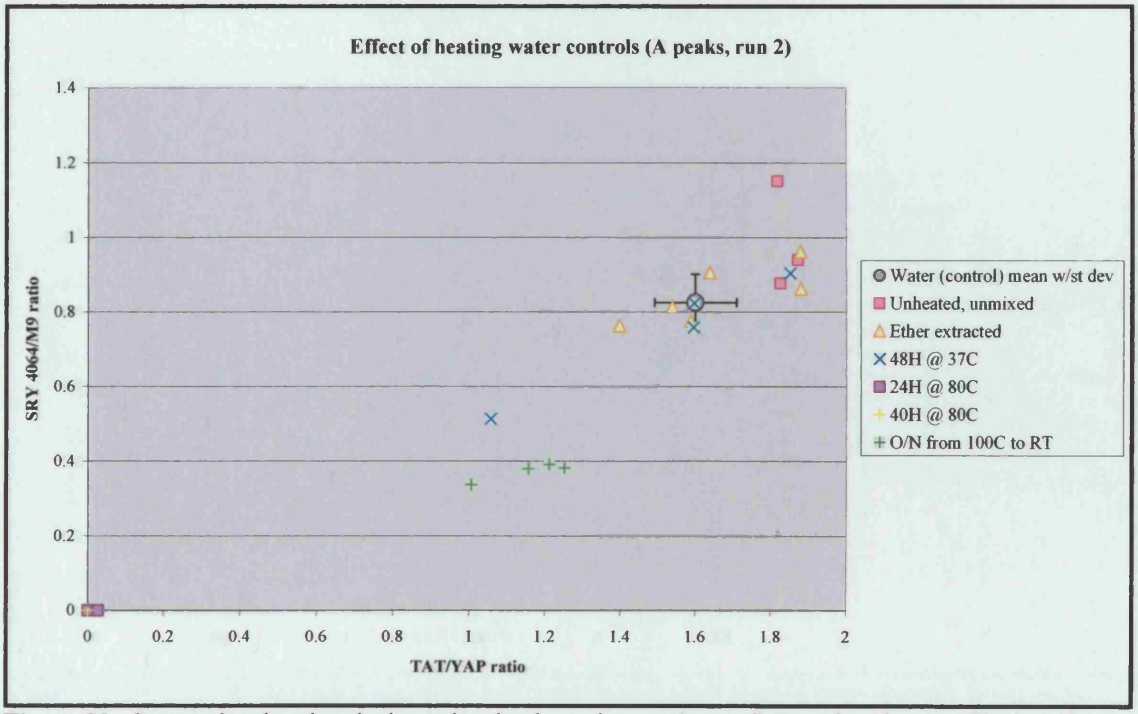


Figure 23. Scatterplot showing the heated and unheated water (control) A peak ratios (run 2), "w/std dev" = with standard deviation, "H" = hours, "O/N" = overnight, "RT" = room temperature. Effects are summarised in Table 12.

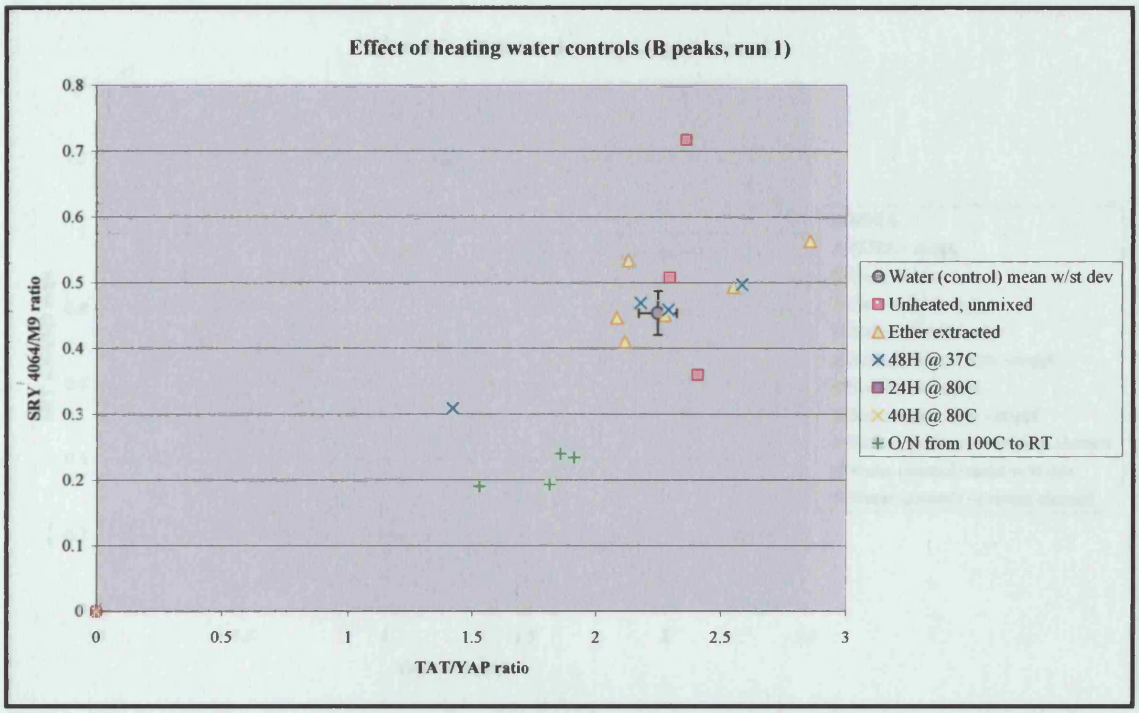


Figure 24. Scatterplot showing the heated and unheated water (control) B peak ratios (run 1), "w/std dev" = with standard deviation, "H" = hours, "O/N" = overnight, "RT" = room temperature. Effects are summarised in Table 12.

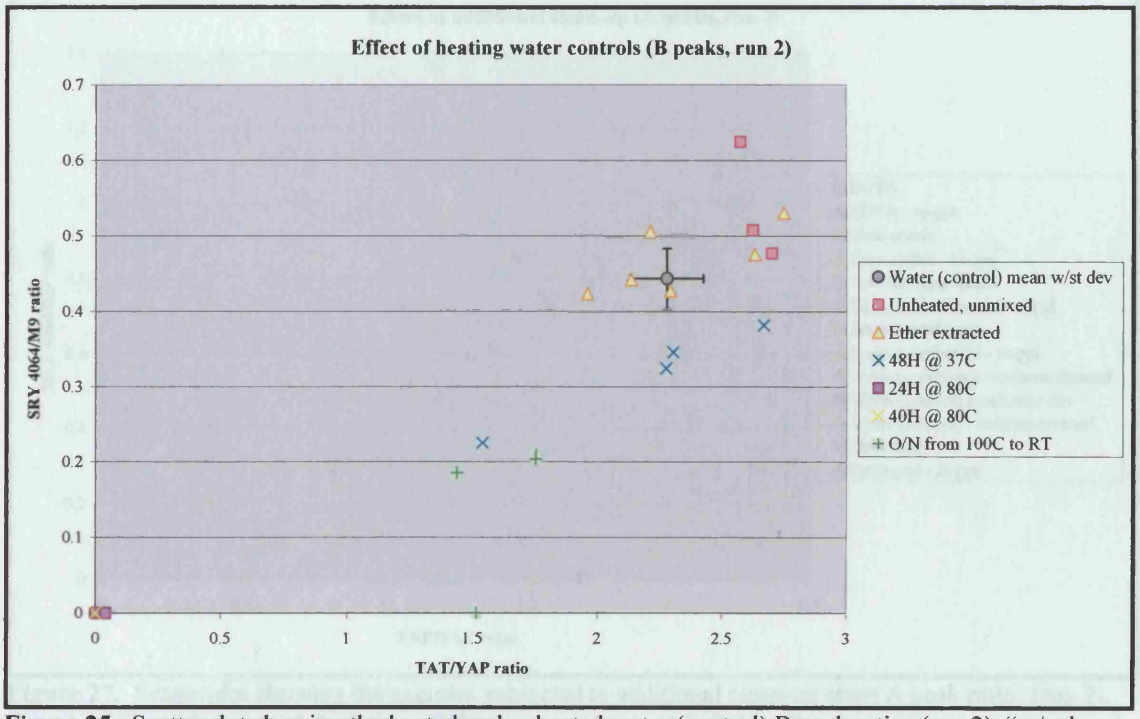


Figure 25. Scatterplot showing the heated and unheated water (control) B peak ratios (run 2), "w/std dev" = with standard deviation, "H" = hours, "O/N" = overnight, "RT" = room temperature. Effects are summarised in Table 12.

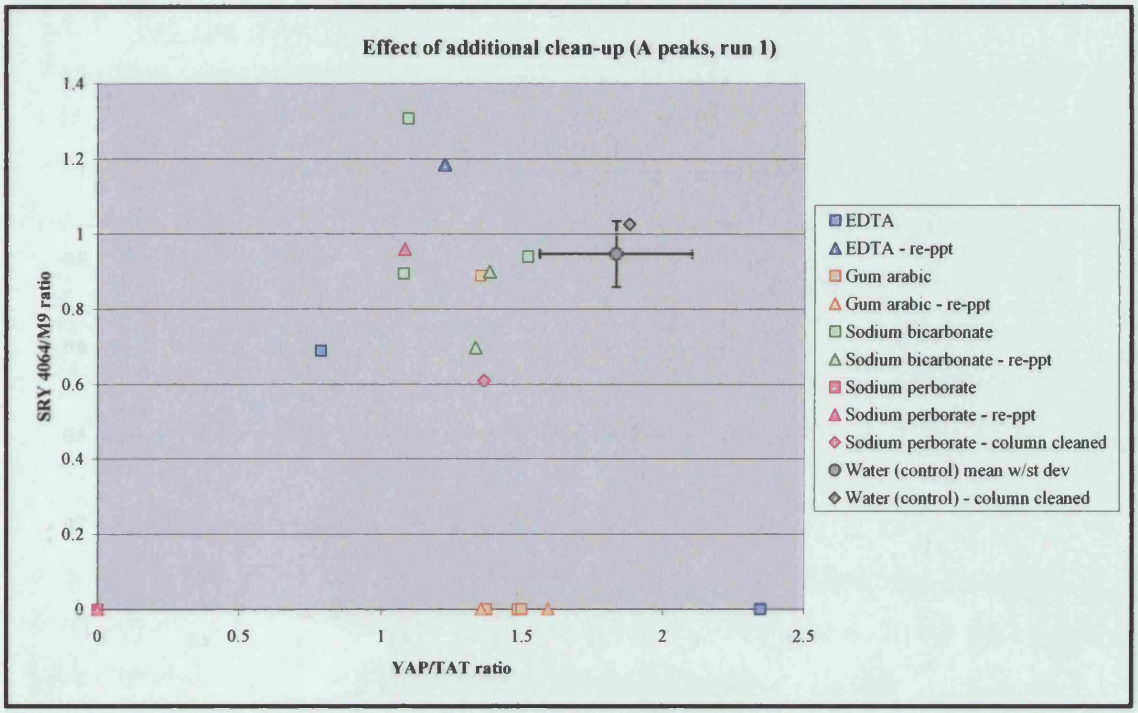


Figure 26. Scatterplot showing the samples subjected to additional clean-up steps A peak ratios (run 1) "w/std dev" = with standard deviation. Effects are summarised in Table 12.

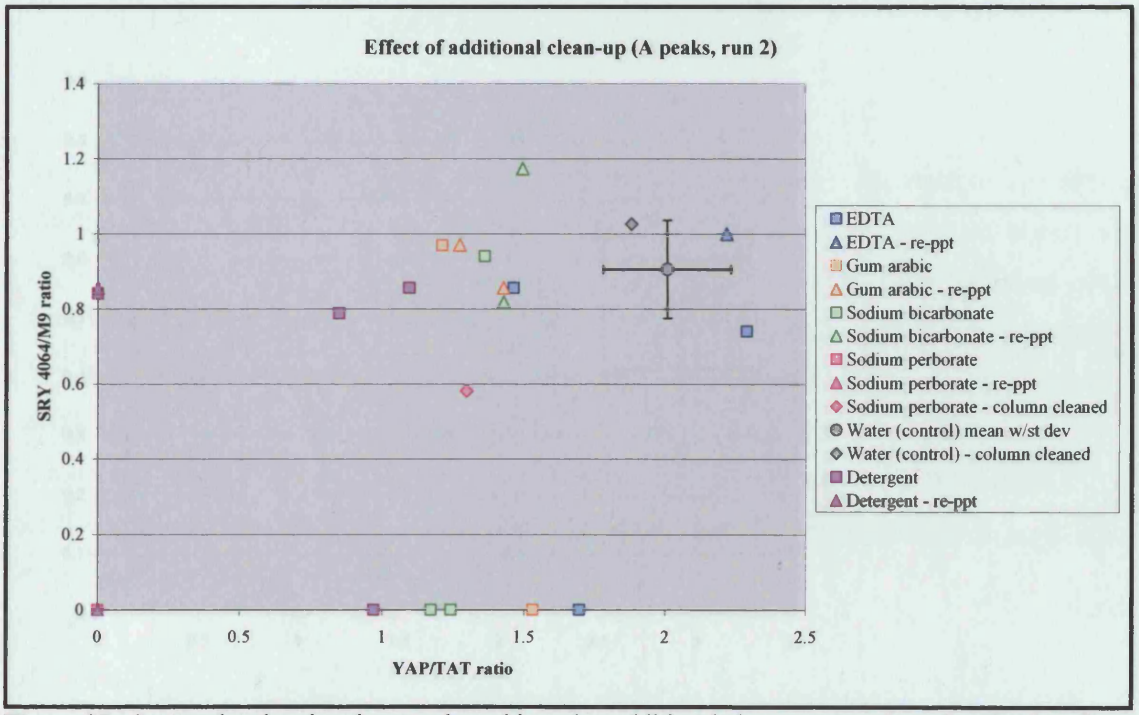


Figure 27. Scatterplot showing the samples subjected to additional clean-up steps A peak ratios (run 2), "w/std dev" = with standard deviation. Effects are summarised in Table 12.

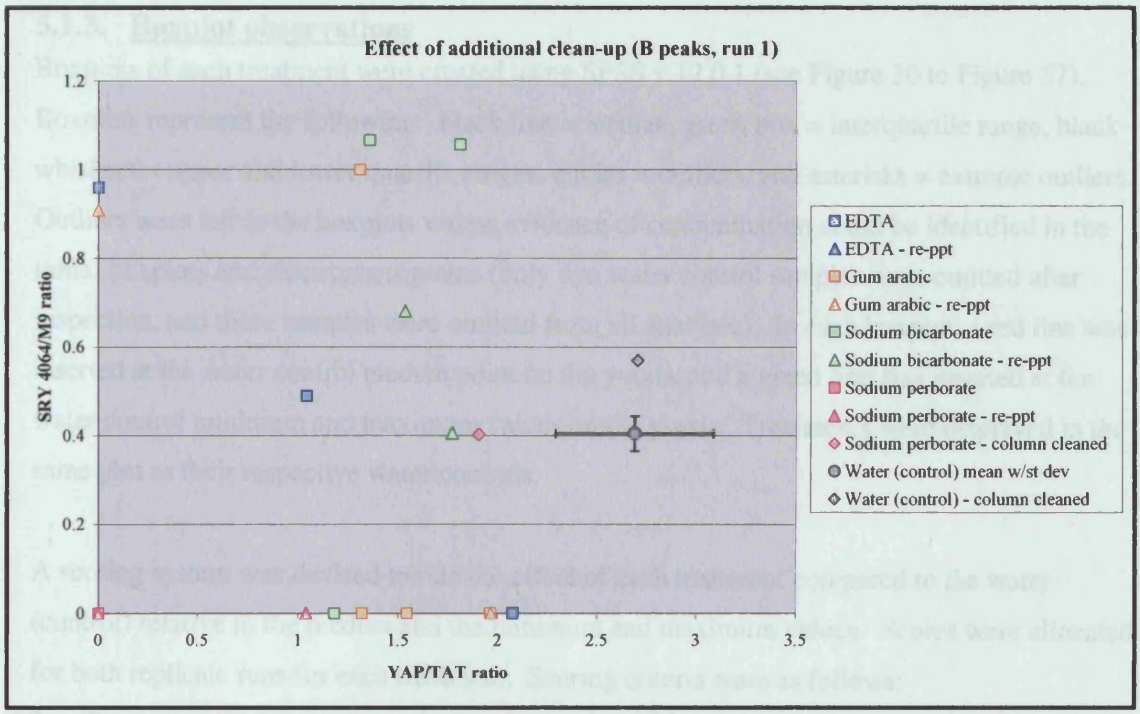


Figure 28. Scatterplot showing the samples subjected to additional clean-up steps B peak ratios (run 1), "w/std dev" = with standard deviation. Effects are summarised in Table 12.

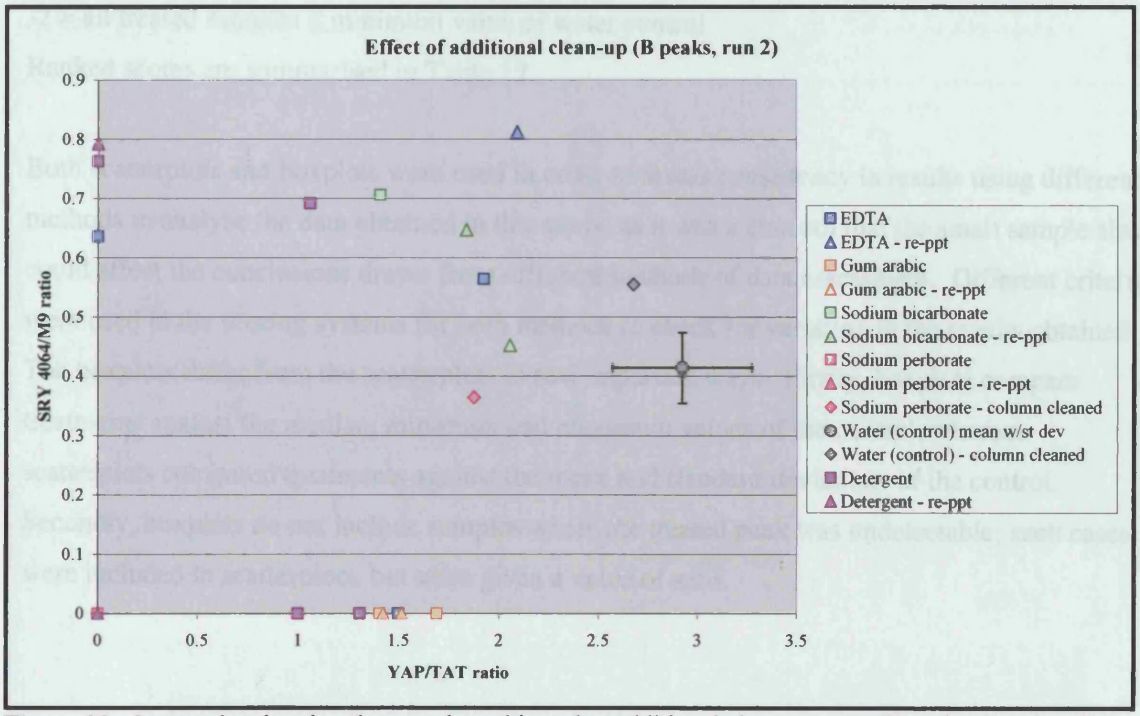


Figure 29. Scatterplot showing the samples subjected to additional clean-up steps B peak ratios (run 2), "w/std dev" = with standard deviation. Effects are summarised in Table 12.

5.1.3. Boxplot observations

Boxplots of each treatment were created using SPSS v.12.0.1 (see Figure 30 to Figure 57). Boxplots represent the following: black line = median, green box = interquartile range, black whiskers = upper and lower quartile ranges, circles = outliers, and asterisks = extreme outliers. Outliers were left in the boxplots unless evidence of contamination could be identified in the initial boxplots and electropherograms (only two water control samples were omitted after inspection, and these samples were omitted from all analyses). In each boxplot, a red line was inserted at the water control median point on the y-axis, and a green line was inserted at the water control minimum and maximum values on the y-axis. Treatments were displayed in the same plot as their respective water controls.

A scoring system was devised to rate the effect of each treatment compared to the water (control) relative to the median and the minimum and maximum values. Scores were allocated for both replicate runs for each treatment. Scoring criteria were as follows:

2 = all treated samples $\geq$ maximum value of water control

1 = all treated samples $\geq$ median of water control

0 = treated samples either side of water control median

-1 = all treated samples $\leq$ median of water control

-2 = all treated samples $\leq$ minimum value of water control

Ranked scores are summarised in Table 12.

Both scatterplots and boxplots were used in order to assess consistency in results using different methods to analyse the data obtained in this study, as it was a concern that the small sample size could affect the conclusions drawn from different methods of data assessment. Different criteria were used in the scoring systems for both methods to check for variation in the results obtained. The boxplots differ from the scatterplots in two important ways. Firstly, boxplots compare treatments against the median, minimum and maximum values of the control, whereas scatterplots compared treatments against the mean and standard deviations of the control. Secondly, boxplots do not include samples where the treated peak was undetectable; such cases were included in scatterplots, but were given a value of zero.

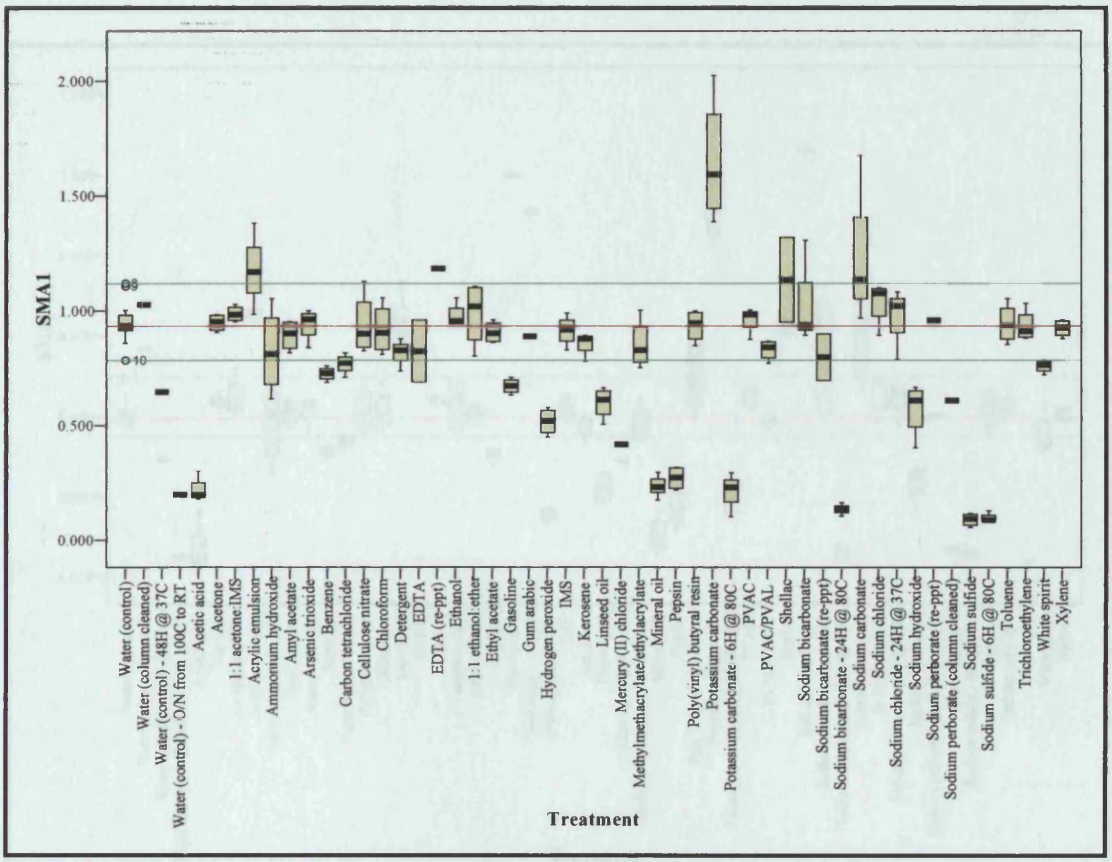


Figure 30. Boxplot comparing the SMA (run 1) peak ratios of treatments with the water control (red line = water control median, green lines = water control minimum and maximum values). Effects of treatments are summarised in Table 12. "H" = hours, "O/N" = overnight, "RT" = room temperature.

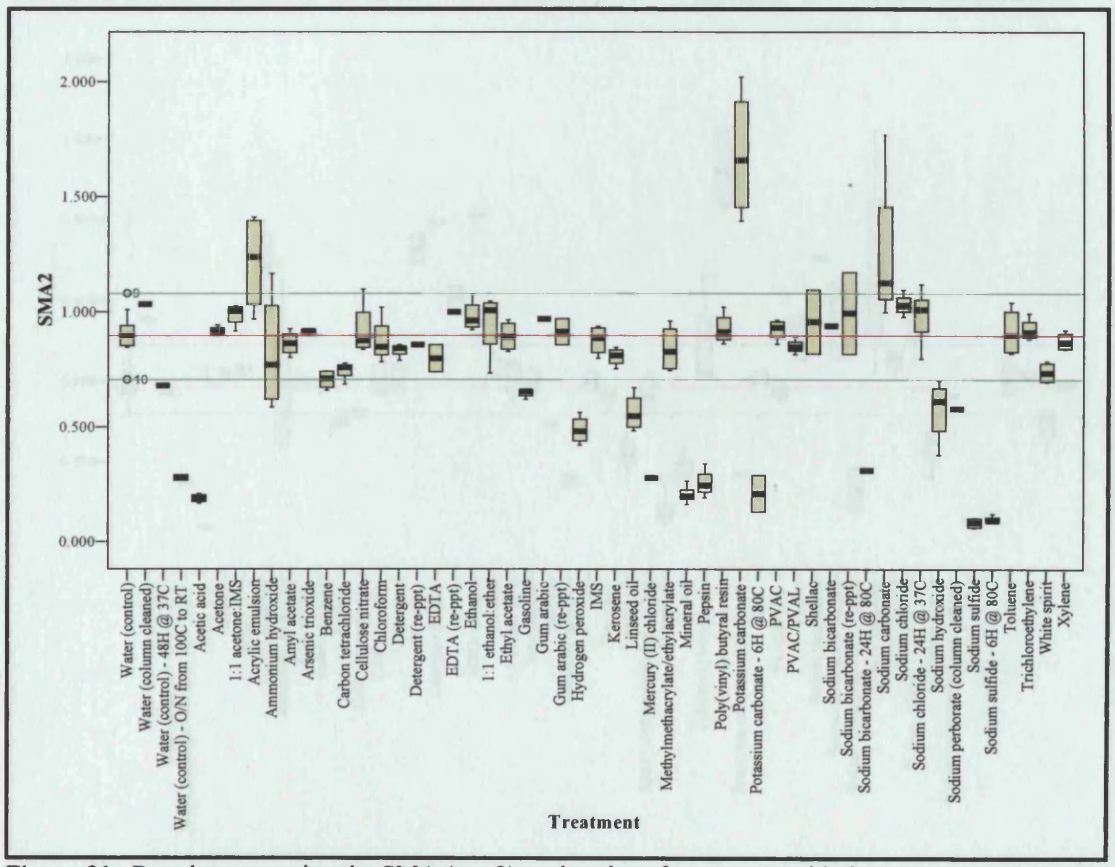


Figure 31. Boxplot comparing the SMA (run 2) peak ratios of treatments with the water control (red line = water control median, green lines = water control minimum and maximum values). Effects of treatments are summarised in Table 12. "H" = hours, "O/N" = overnight, "RT" = room temperature.

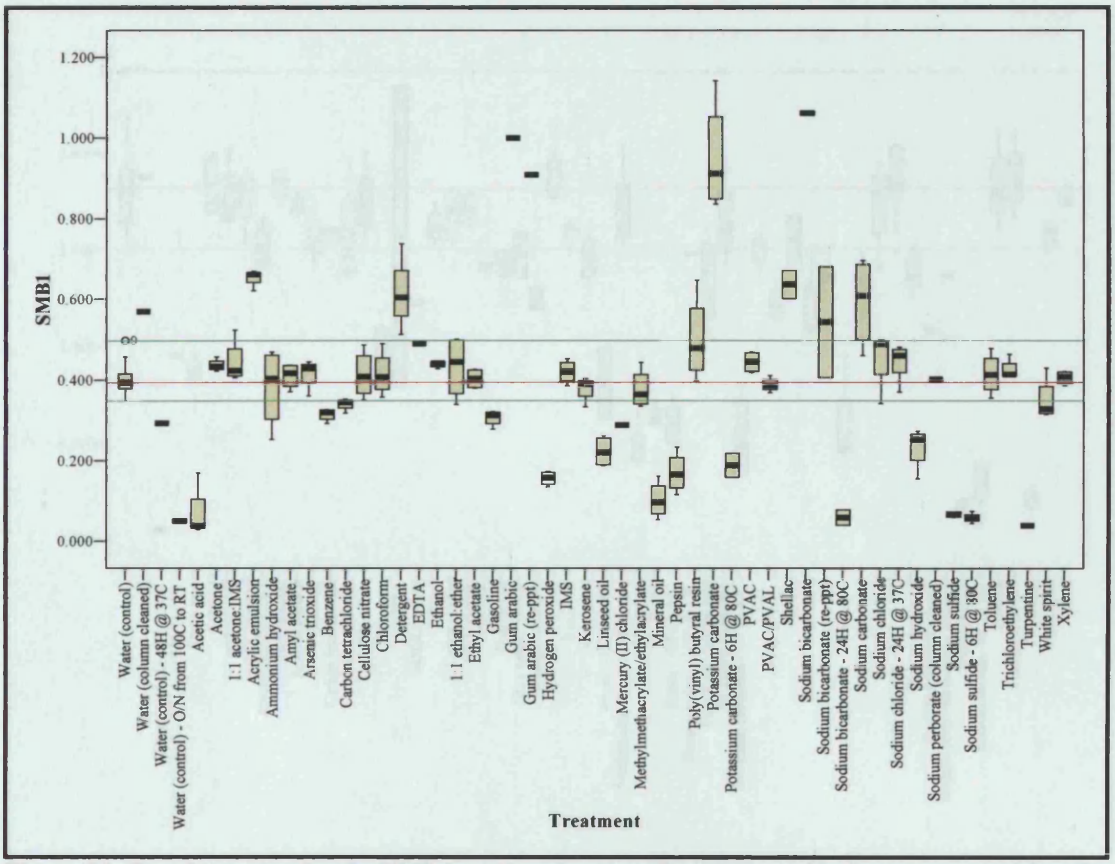


Figure 32. Boxplot comparing the SMB (run 1) peak ratios of treatments with the water control (red line = water control median, green lines = water control minimum and maximum values). Effects of treatments are summarised in Table 12. "H" = hours, "O/N" = overnight, "RT" = room temperature.

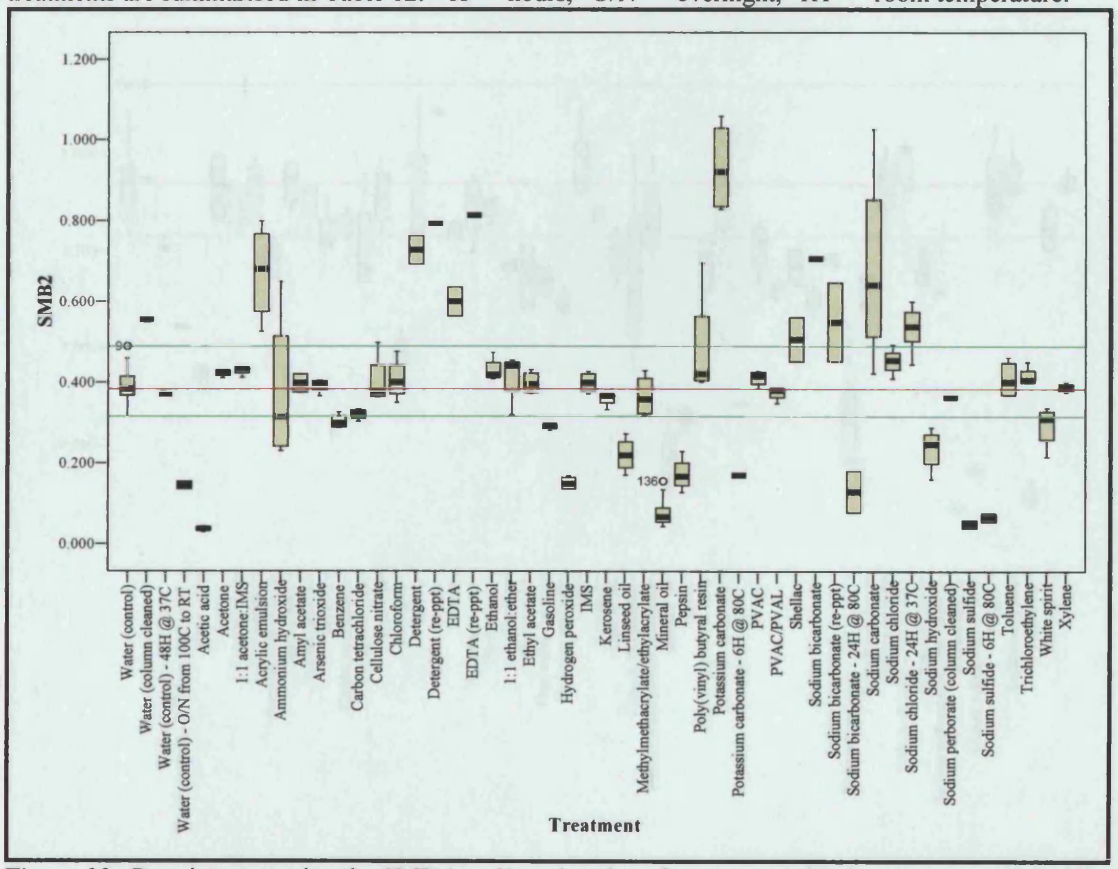


Figure 33. Boxplot comparing the SMB (run 2) peak ratios of treatments with the water control (red line = water control median, green lines = water control minimum and maximum values). Effects of treatments are summarised in Table 12. "H" = hours, "O/N" = overnight, "RT" = room temperature.

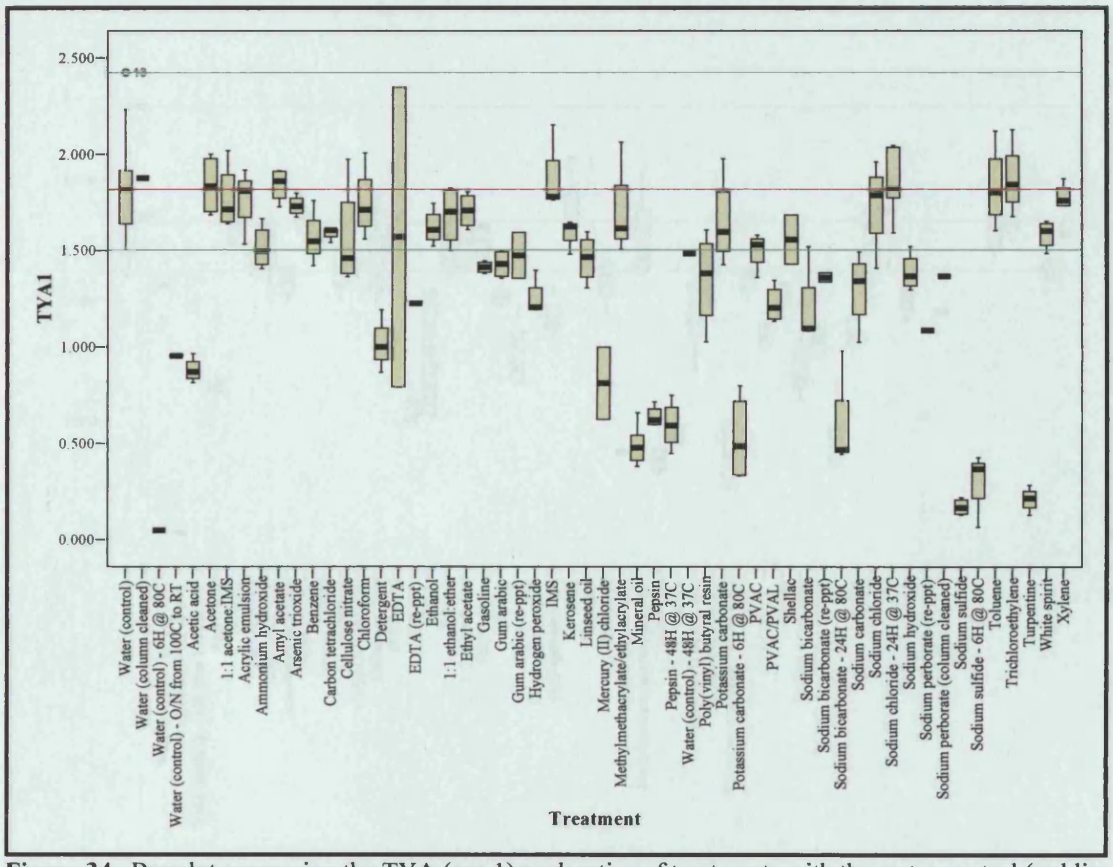


Figure 34. Boxplot comparing the TYA (run 1) peak ratios of treatments with the water control (red line = water control median, green lines = water control minimum and maximum values). Effects of treatments are summarised in Table 12. "H" = hours, "O/N" = overnight, "RT" = room temperature.

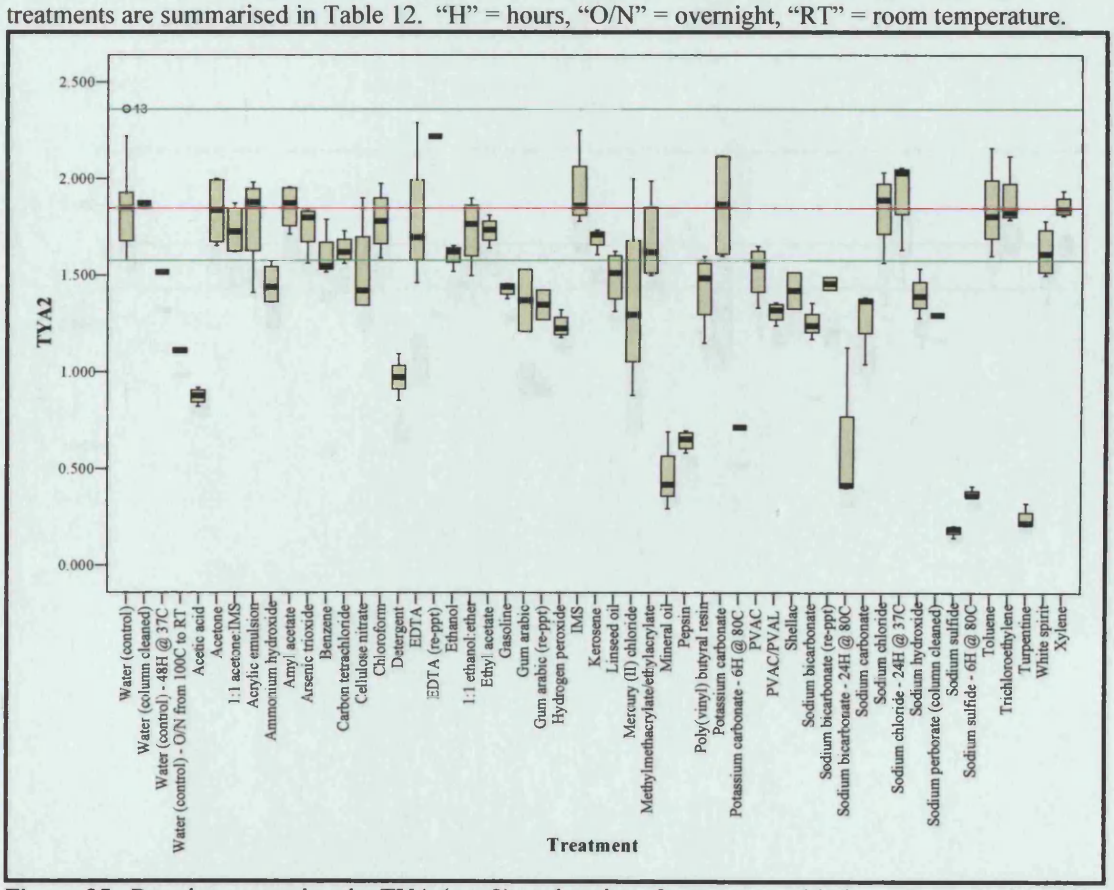


Figure 35. Boxplot comparing the TYA (run 2) peak ratios of treatments with the water control (red line = water control median, green lines = water control minimum and maximum values). Effects of treatments are summarised in Table 12. "H" = hours, "O/N" = overnight, "RT" = room temperature.

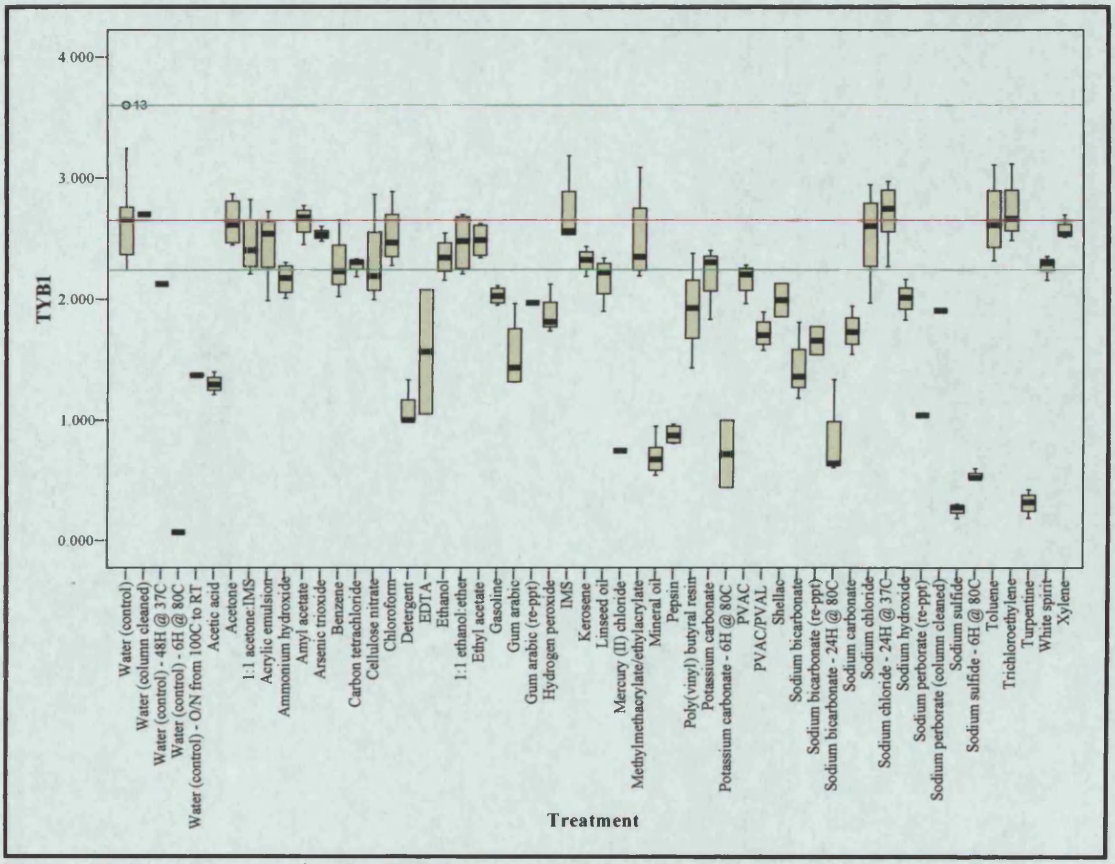


Figure 36. Boxplot comparing the TYB (run 1) peak ratios of treatments with the water control (red line = water control median, green lines = water control minimum and maximum values). Effects of treatments are summarised in Table 12. "H" = hours, "O/N" = overnight, "RT" = room temperature.

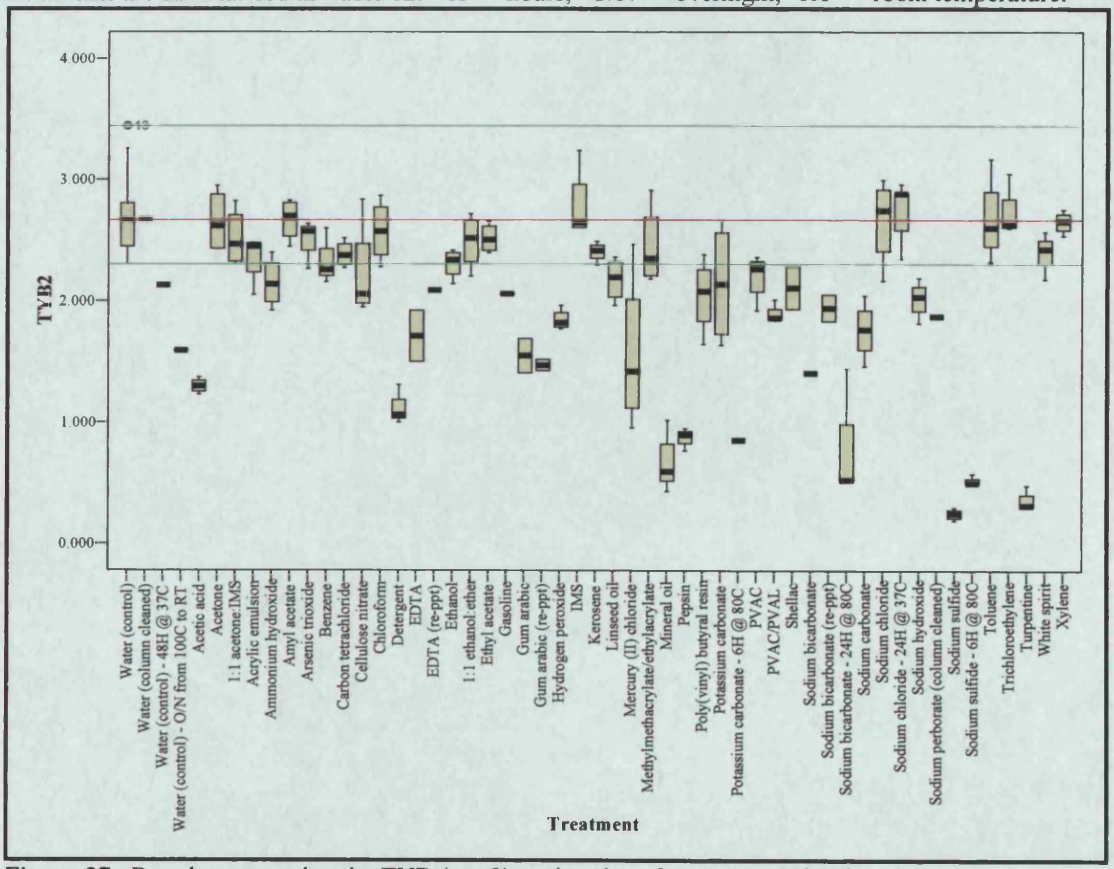


Figure 37. Boxplot comparing the TYB (run 2) peak ratios of treatments with the water control (red line = water control median, green lines = water control minimum and maximum values). Effects of treatments are summarised in Table 12. "H" = hours, "O/N" = overnight, "RT" = room temperature.

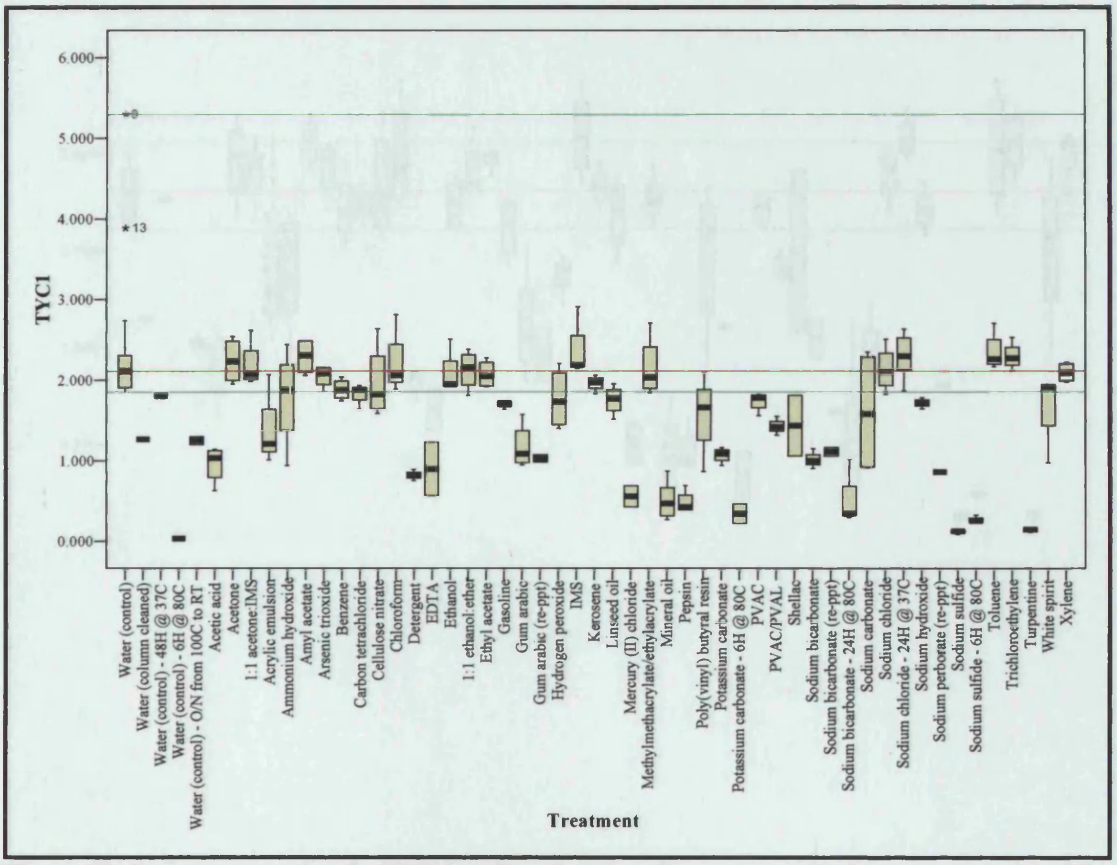


Figure 38. Boxplot comparing the TYC (run 1) peak ratios of treatments with the water control (red line = water control median, green lines = water control minimum and maximum values). Effects of treatments are summarised in Table 12. "H" = hours, "O/N" = overnight, "RT" = room temperature.

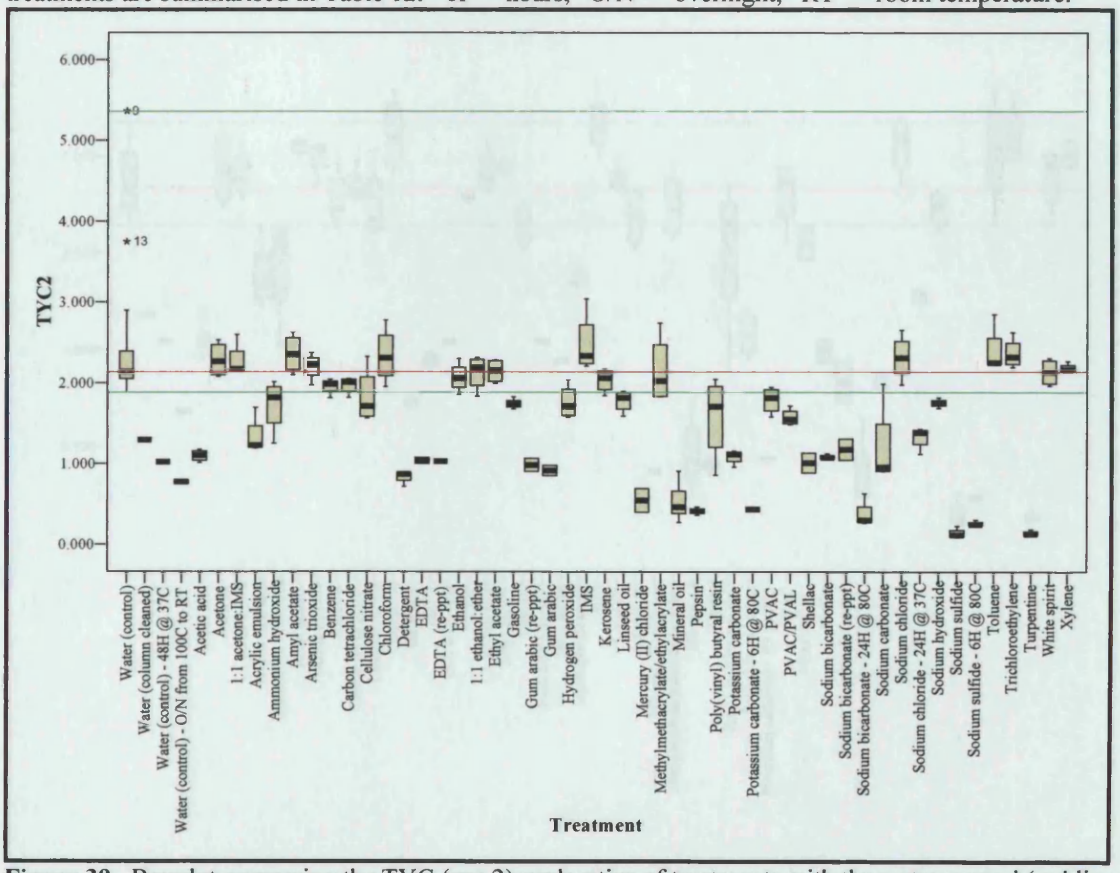


Figure 39. Boxplot comparing the TYC (run 2) peak ratios of treatments with the water control (red line = water control median, green lines = water control minimum and maximum values). Effects of treatments are summarised in Table 12. "H" = hours, "O/N" = overnight, "RT" = room temperature.

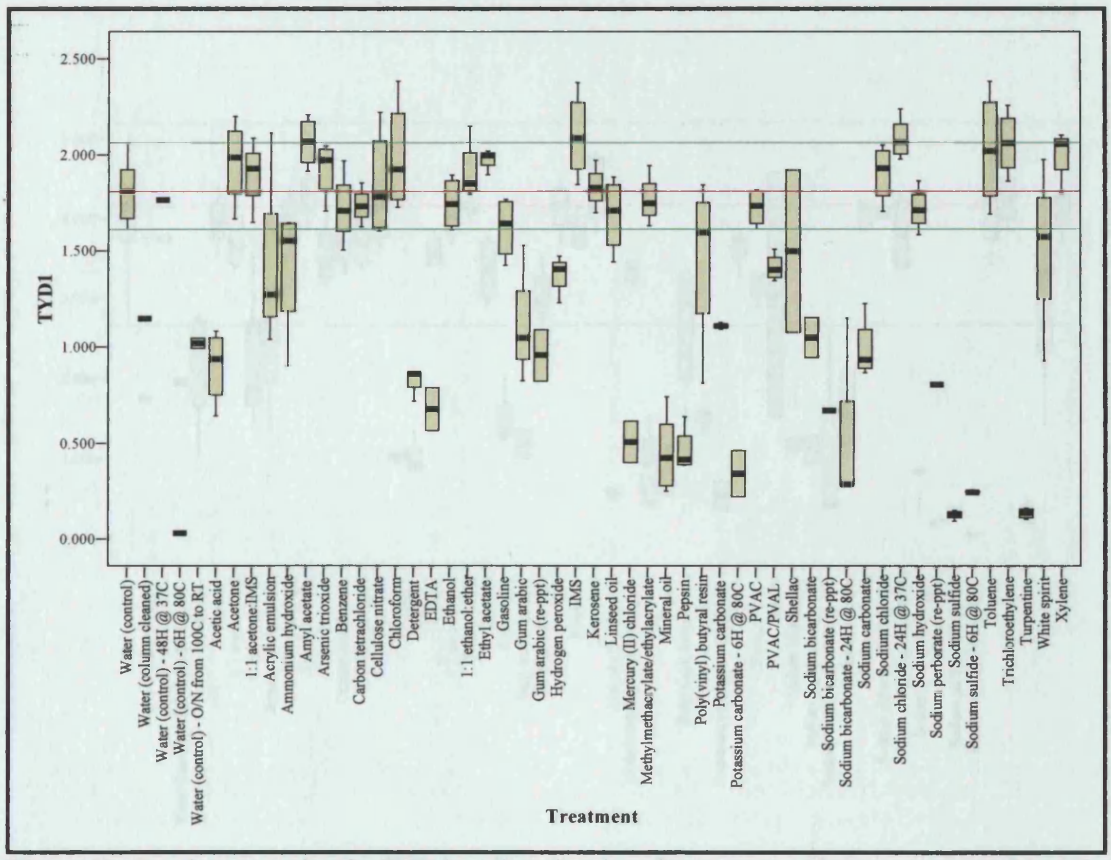


Figure 40. Boxplot comparing the TYD (run 1) peak ratios of treatments with the water control (red line = water control median, green lines = water control minimum and maximum values). Effects of treatments are summarised in Table 12. "H" = hours, "O/N" = overnight, "RT" = room temperature.

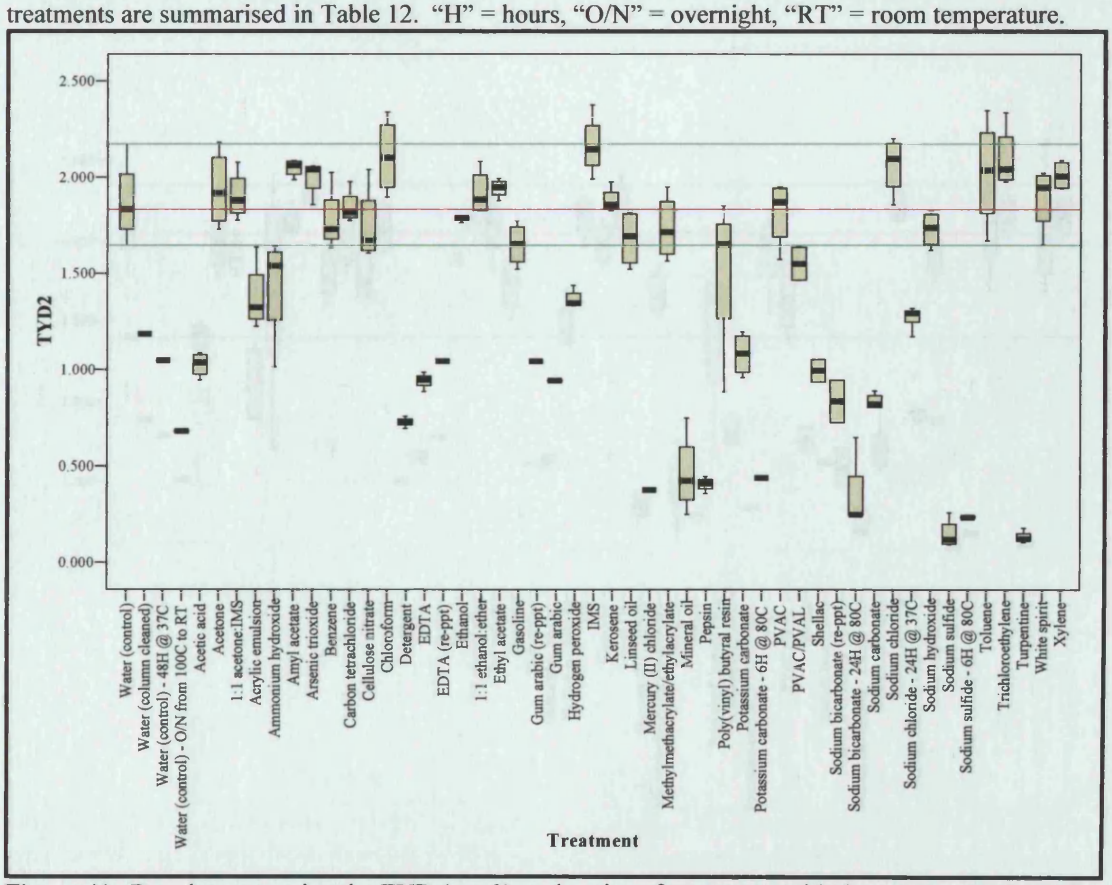


Figure 41. Boxplot comparing the TYD (run 2) peak ratios of treatments with the water control (red line = water control median, green lines = water control minimum and maximum values). Effects of treatments are summarised in Table 12. "H" = hours, "O/N" = overnight, "RT" = room temperature.

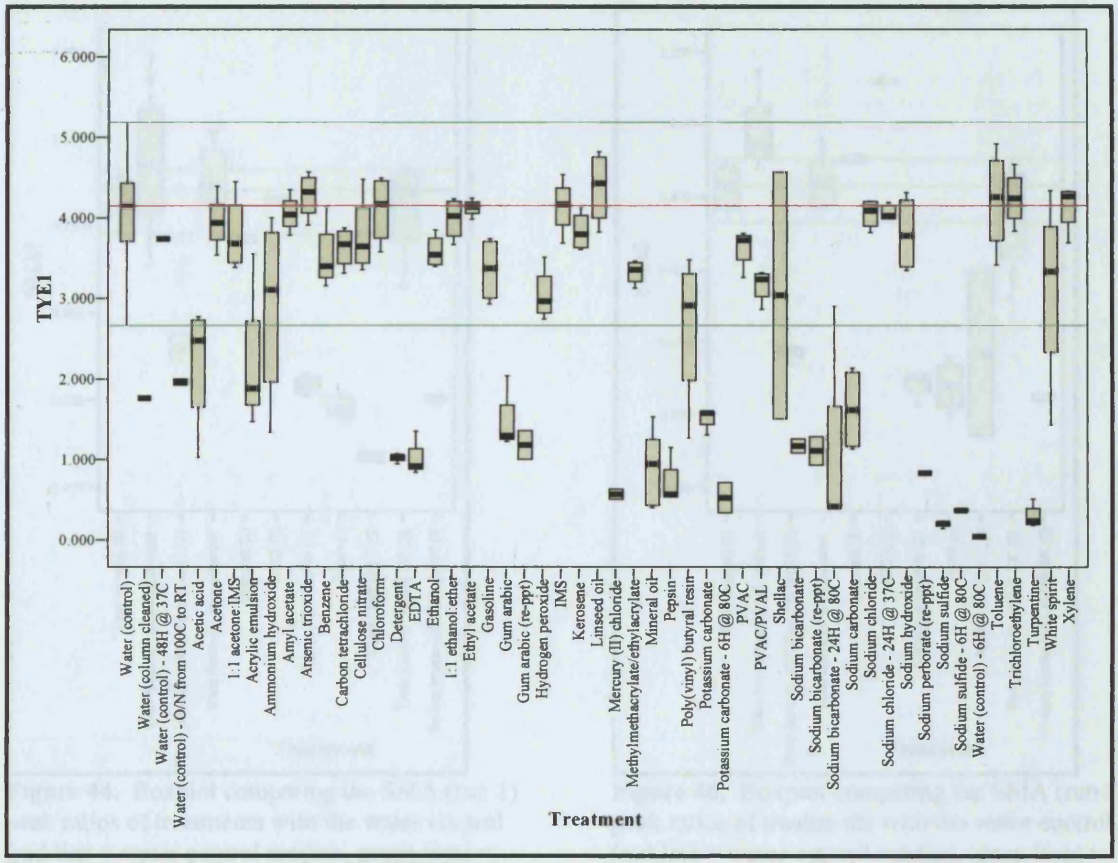


Figure 42. Boxplot comparing the TYE (run 1) peak ratios of treatments with the water control (red line = water control median, green lines = water control minimum and maximum values). Effects of treatments are summarised in Table 12. "H" = hours, "O/N" = overnight, "RT" = room temperature.

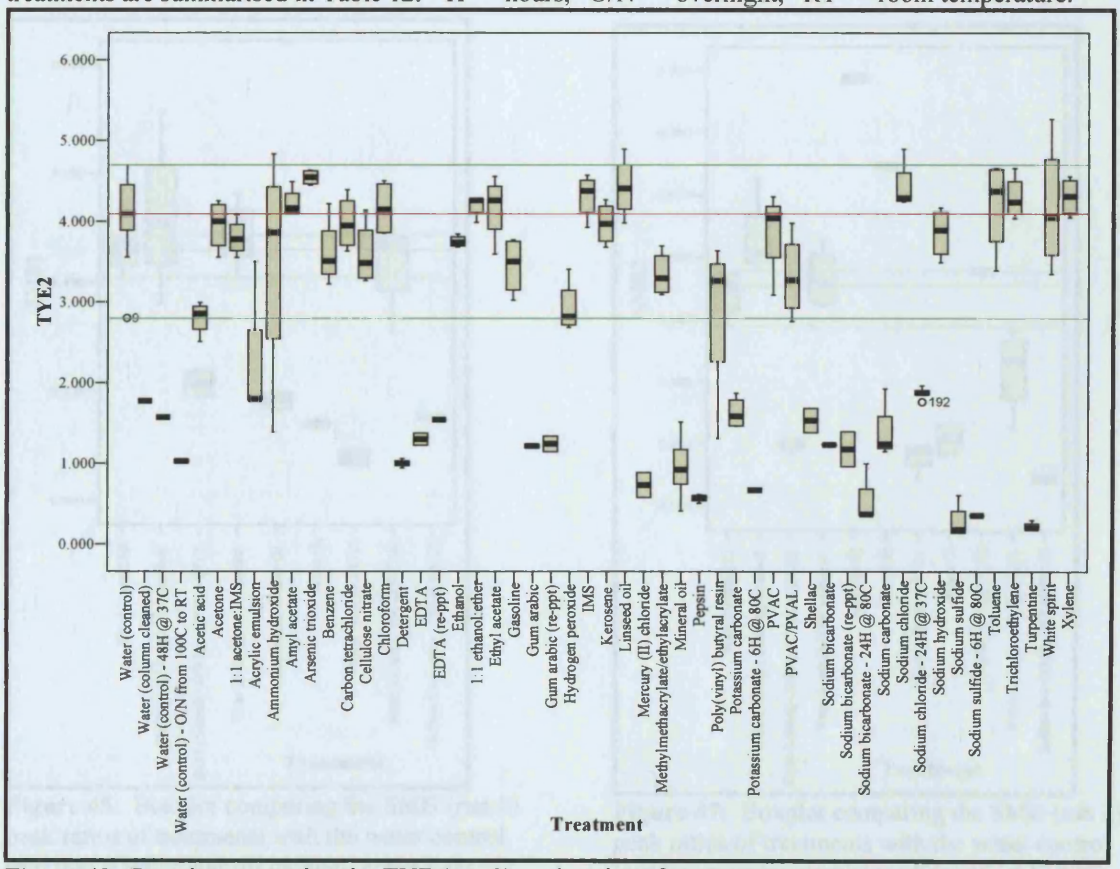


Figure 43. Boxplot comparing the TYE (run 2) peak ratios of treatments with the water control (red line = water control median, green lines = water control minimum and maximum values). Effects of treatments are summarised in Table 12. "H" = hours, "O/N" = overnight, "RT" = room temperature.

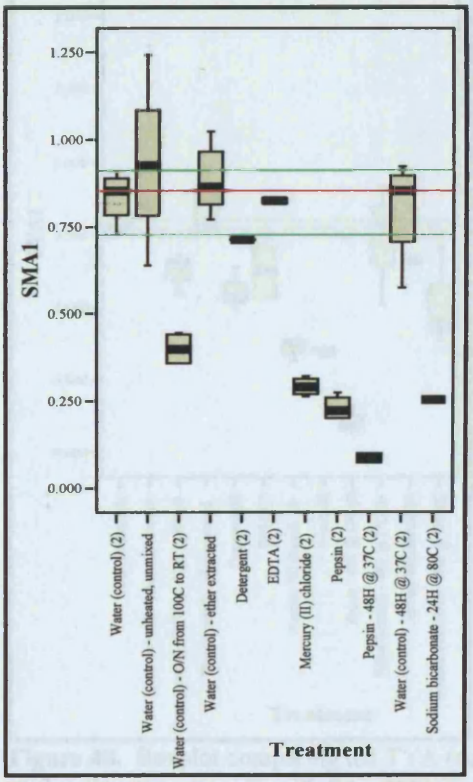


Figure 44. Boxplot comparing the SMA (run 1) peak ratios of treatments with the water control (red line = water control median, green lines = water control minimum and maximum values). Treatment effects are summarised in Table 12.

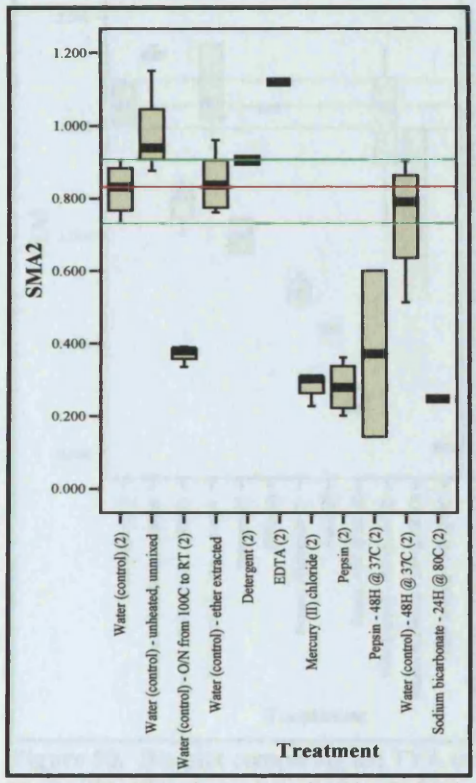


Figure 46. Boxplot comparing the SMA (run 2) peak ratios of treatments with the water control (red line = water control median, green lines = water control minimum and maximum values). Treatment effects are summarised in Table 12.

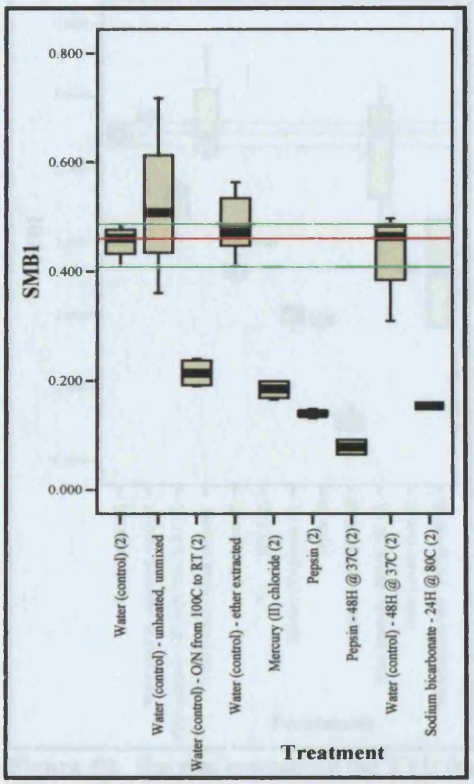


Figure 45. Boxplot comparing the SMB (run 1) peak ratios of treatments with the water control (red line = water control median, green lines = water control minimum and maximum values). Treatment effects are summarised in Table 12.

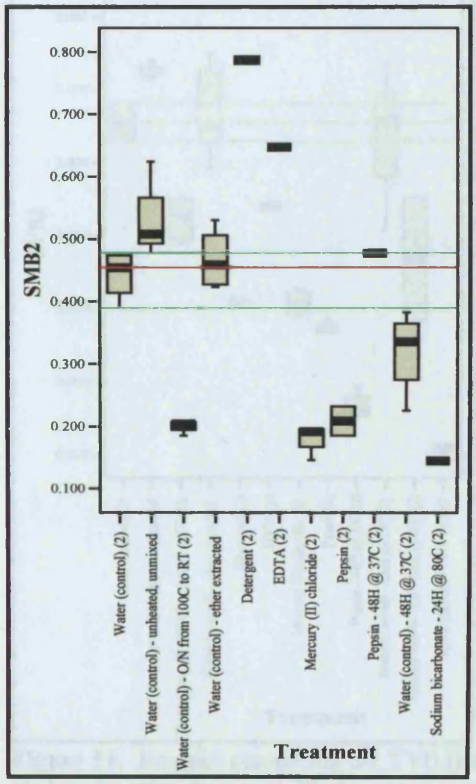


Figure 47. Boxplot comparing the SMB (run 2) peak ratios of treatments with the water control (red line = water control median, green lines = water control minimum and maximum values). Treatment effects are summarised in Table 12.

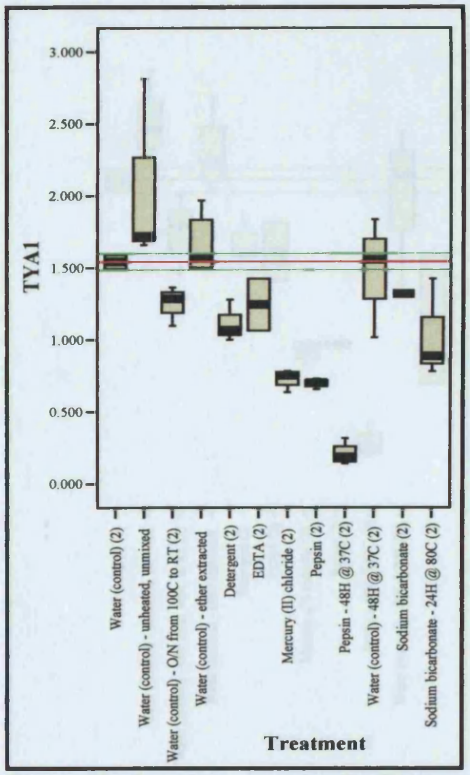


Figure 48. Boxplot comparing the TYA (run 1) peak ratios of treatments with the water control (red line = water control median, green lines = water control minimum and maximum values). Treatment effects are summarised in Table 12.

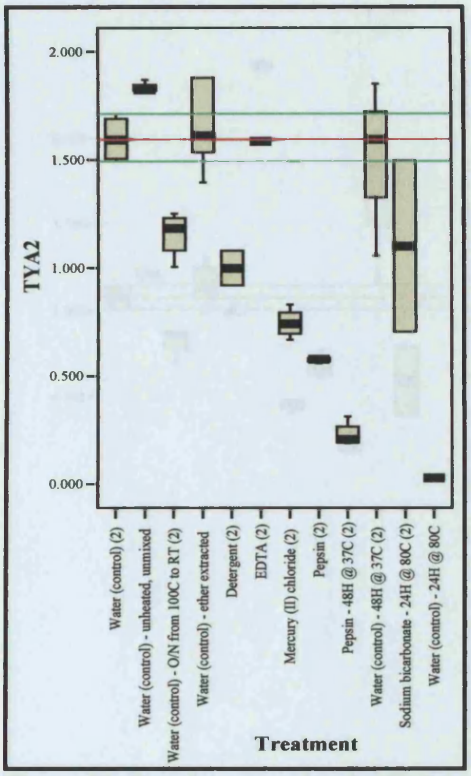


Figure 50. Boxplot comparing the TYA (run 2) peak ratios of treatments with the water control (red line = water control median, green lines = water control minimum and maximum values). Treatment effects are summarised in Table 12.

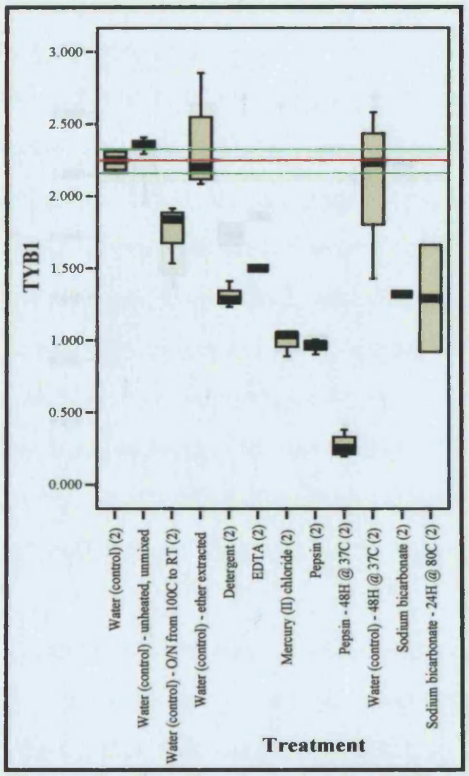


Figure 49. Boxplot comparing the TYB (run 1) peak ratios of treatments with the water control (red line = water control median, green lines = water control minimum and maximum values). Treatment effects are summarised in Table 12.

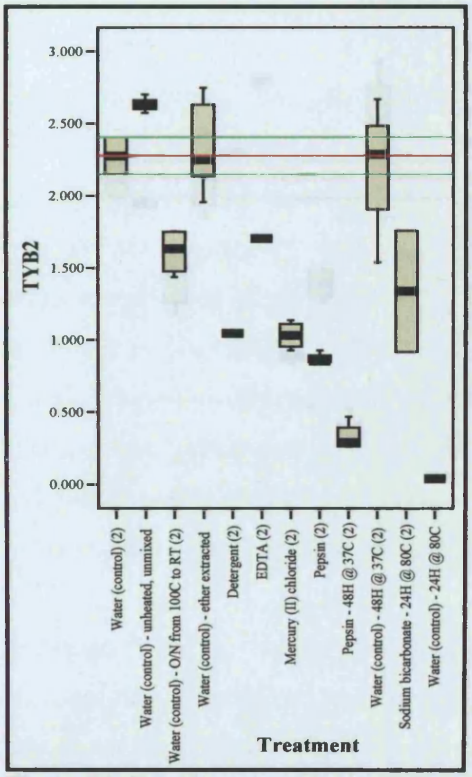


Figure 51. Boxplot comparing the TYB (run 2) peak ratios of treatments with the water control (red line = water control median, green lines = water control minimum and maximum values). Treatment effects are summarised in Table 12.

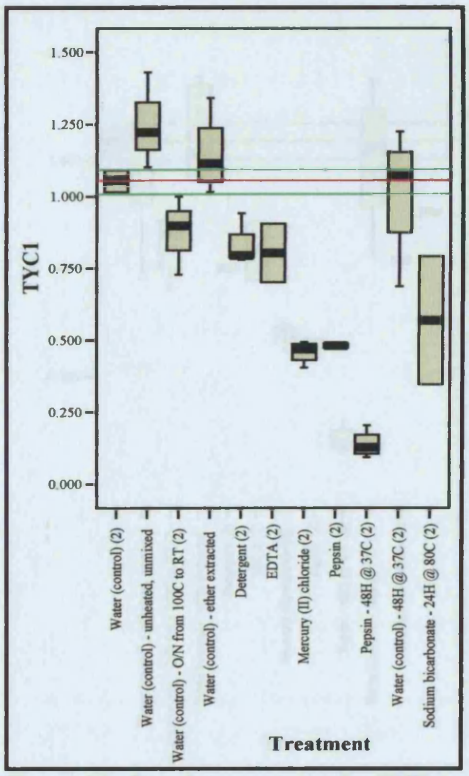


Figure 52. Boxplot comparing the TYC (run 1) peak ratios of treatments with the water control (red line = water control median, green lines = water control minimum and maximum values). Treatment effects are summarised in Table 12.

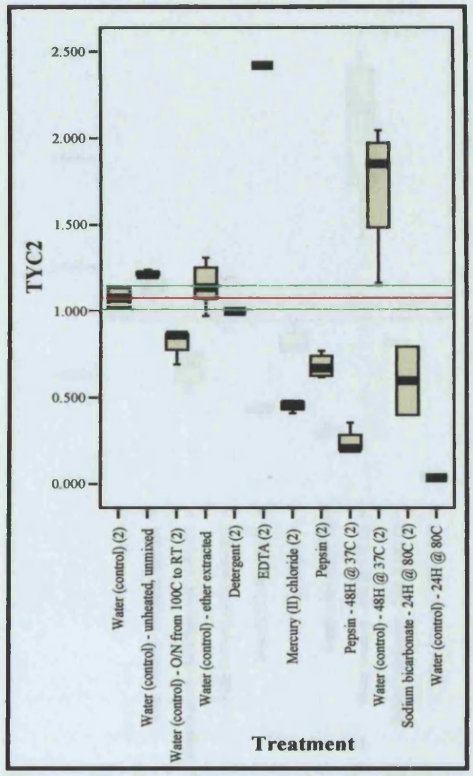


Figure 54. Boxplot comparing the TYC (run 2) peak ratios of treatments with the water control (red line = water control median, green lines = water control minimum and maximum values). Treatment effects are summarised in Table 12.

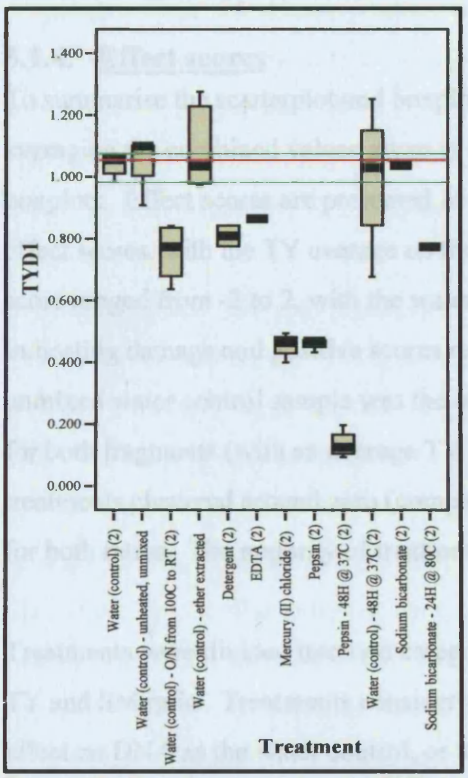


Figure 53. Boxplot comparing the TYD (run 1) peak ratios of treatments with the water control (red line = water control median, green lines = water control minimum and maximum values). Treatment effects are summarised in Table 12.

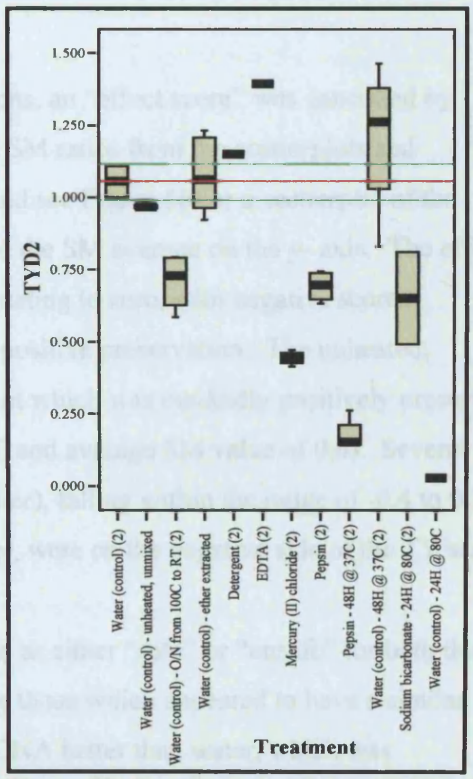


Figure 55. Boxplot comparing the TYD (run 2) peak ratios of treatments with the water control (red line = water control median, green lines = water control minimum and maximum values). Treatment effects are summarised in Table 12.

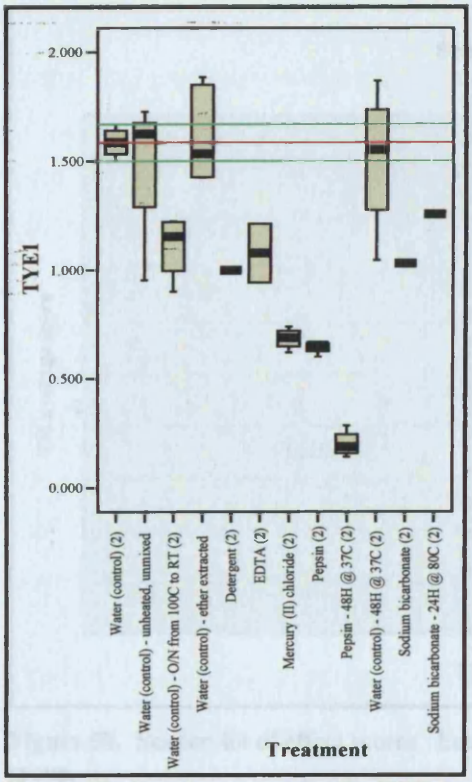


Figure 56. Boxplot comparing the TYE (run 1) peak ratios of treatments with the water control (red line = water control median, green lines = water control minimum and maximum values). Treatment effects are summarised in Table 12.

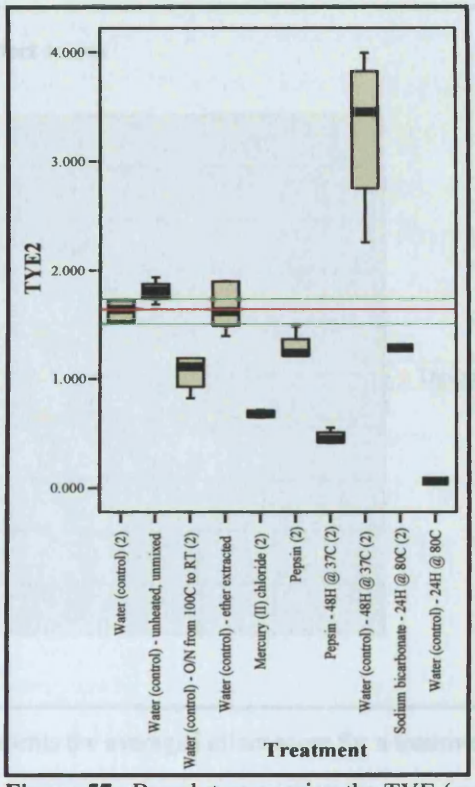


Figure 57. Boxplot comparing the TYE (run 2) peak ratios of treatments with the water control (red line = water control median, green lines = water control minimum and maximum values). Treatment effects are summarised in Table 12.

5.1.4. Effect scores

To summarise the scatterplot and boxplot observations, an "effect score" was generated by averaging the combined values given to the TY and SM ratios from the scatterplots and boxplots. Effect scores are presented in Table 12, and see Figure 58 for a scatterplot of the effect scores, with the TY average on the x- axis and the SM average on the y- axis. The effect score ranged from -2 to 2, with the water control equating to zero, with negative scores indicating damage and positive scores representing positive preservation. The unheated, unmixed water control sample was the only treatment which was markedly positively preserved for both fragments (with an average TY value of 1.2 and average SM value of 0.6). Several treatments clustered around zero (comparable to water), falling within the range of -0.4 to 0.5 for both ratios. The majority of treatments, however, were on the negative side of the TY scale.

Treatments were divided into two categories defined as either "safe" or "unsafe" for both the TY and SM ratio. Treatments considered safe, were those which appeared to have a similar effect on DNA as the water control, or to preserve DNA better than water, which was determined to be a value equal to or greater than -0.4, as some variation around zero should be expected and tolerated. Unsafe treatments were those which appeared damaging to DNA and had an effect score of less than -0.4.

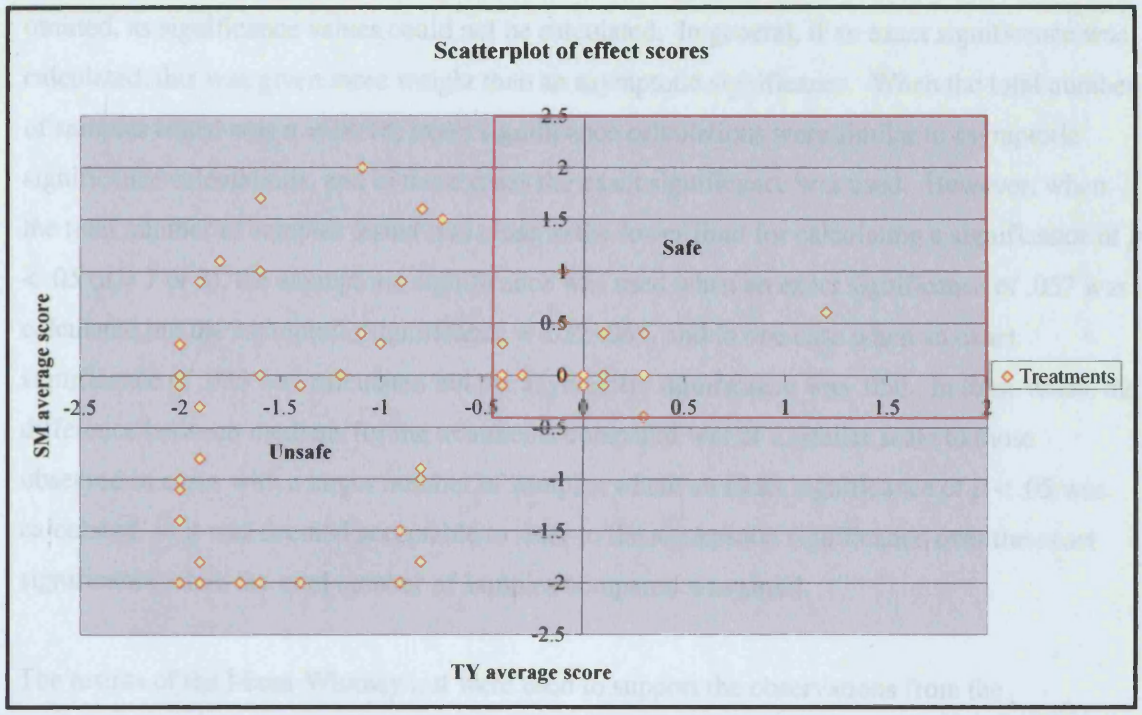


Figure 58. Scatterplot of effect scores. Each point represents the averaged effect score for a treatment group.

5.1.5. Mann-Whitney test

Mann-Whitney U scores and significance values were calculated using SPSS to assess the peak height ratio data. The Mann-Whitney test was chosen because it provides significance values based on the comparison of two groups (e.g. treatments or a treatment and the control), and is suitable for non-parametric data. A two-tailed significance was calculated, as no assumptions were made as to whether any particular treatments would be more or less damaging than the water control. Again, only the A and B peaks were used for this test.

The significance value calculated was used to determine whether the two groups compared were from the same population. The difference between the two groups was statistically significant if $p \leq .05$. In order to determine which group was more preserving, the median values were used. DNA was better preserved in the group with a higher median peak height value.

In addition to comparing all treatments with their respective water controls, statistics were also calculated for any other relevant comparisons, such as organic solvent-based treatments compared to the organic solvent alone, and heated treatments compared to unheated treatments. Mann-Whitney U statistics were calculated for both runs of each peak, resulting in a total of up to 14 statistics calculated for each pair of compared treatments. Some peaks or treatments were necessarily omitted from Mann-Whitney test assessment due to small sample size limitations inherent in this test. Cases where data were available for less than two samples for any single peak were omitted, as significance values of $p < .05$ could not be calculated. Similarly, cases where data was available for a total of less than seven samples for any given comparison were

omitted, as significance values could not be calculated. In general, if an exact significance was calculated, this was given more weight than an asymptotic significance. When the total number of samples tested was n = 14-18, exact significance calculations were similar to asymptotic significance calculations, and in these cases the exact significance was used. However, when the total number of samples tested was close to the lower limit for calculating a significance of $p < .05$ (n = 7 or 8), the asymptotic significance was used when an exact significance of .057 was calculated but the asymptotic significance = .032-.043, and in one case when an exact significance of .086 was calculated but the asymptotic significance was .050. In these cases, the difference between medians for the treatments compared was of a similar scale to those observed in cases with a larger number of samples where an exact significance of $p < .05$ was calculated, so it was deemed acceptable to refer to the asymptotic significance over the exact significance when the total number of samples compared was small.

The results of the Mann-Whitney test were used to support the observations from the scatterplots and boxplots, by confirming if the differences between treatments observed using the other methods of assessment were statistically significant. The Mann-Whitney test was carried out for all peak height ratios, and for both sample runs (a total of 14 comparisons per treatment, given a full dataset). Effects of treatments were assessed separately for the 100bp (SMA1-SMB2) and 200bp (TYA1-TYE2) fragments. For each set of fragments where statistics were calculated, the suitability of treatments was assessed as follows:
"Unsafe" = the Mann-Whitney U value was small, and $p \leq .05$ for any of the statistics calculated, and for the significant statistics, the median for the water control was larger than the median for the treatment
"Safe" = the Mann-Whitney U value was large, and $p \leq .05$ for none of the statistics calculated OR the Mann-Whitney U value was small, and $p \leq .05$ for any of the statistics calculated, and for the significant statistics, the median for the treatment was larger than the median for the control

Where two treated groups (e.g. sodium chloride and sodium chloride heated to 37°C for 24 hours) were compared with each other, one group was deemed to be "unsafe compared" to the other group if the $p \leq .05$, and its median was lower than the other group. If the $p > .05$, the two groups were deemed to be "comparable".

In the majority of cases where $p \leq .05$ for more than one statistic in a treatment, the results were in agreement with each other (i.e. all were either safe or unsafe). However, in a few cases, there were inconsistencies in the results, with a single statistic being significantly different from the rest. This happened for the TYD2 statistic for water (control) – unheated, unmixed; for the SMA1 statistic for detergent; for the SMB1 statistic for sodium bicarbonate; and the TYD1

statistic for sodium chloride heated to 37°C for 24 hours. For each of these treatments, at least three significant statistics were calculated, therefore the final determination of safe or unsafe was based on the majority of cases, and the odd statistic was ignored.

All statistical calculations are shown in Appendix B. A summary of the effects is provided in Table 12.

Treatment	Scatterplot observations								Boxplot observations														Effect score		Plot conclusion		Mann-Whitney conclusion	
	2 = all samples ≥ control standard deviation; 1 = all samples ≥ control mean, 0 = samples above and below control mean, -1 = all samples ≤ control mean, -2 = all samples ≤ control standard deviation								*2 = treated samples above maximum value of water control, 1 = treated samples above median of water control, 0 = treated samples included median of water control, -1 = treated samples below median of water control, -2 = treated samples below minimum value of water control)*														*TY ave = mean of all TY scores; SM ave = mean of all SM scores*		*Safe = x ≥ -0.4; Unsafe = x < -0.4*		*Unsafe = small U value, and at least one p < .05, and median of treatment less than control; otherwise, safe*	
	TYA1	TYA2	TYB1	TYB2	SMA1	SMA2	SMB1	SMB2	TYA1	TYA2	TYB1	TYB2	TYC1	TYC2	TYD1	TYD2	TYE1	TYE2	SMA1	SMA2	SMB1	SMB2	TY ave	SM ave	TY	SM	TY	SM
Water (control) - column cleaned	1	-1	0	-1	1	1	2	2	1	1	1	0	-2	-2	-2	-2	-2	-2	1	1	2	2	-0.7	1.5	Unsafe	Safe		
Water (control) - 48H @ 37°C	-2	-2	-2	-2	-2	-2	-2	-1	-2	-2	-2	-2	-2	-2	-1	-2	-1	-2	-2	-2	-2	-1	-1.9	-1.8	Unsafe	Unsafe		
Water (control) - 6H @ 80°C	-2	-2	-2	-2	-2	-2	-2	-2	-2		-2		-2		-2		-2						-2.0	-2.0	Unsafe	Unsafe		
Water (control) - 40H @ 80°C	-2	-2	-2	-2	-2	-2	-2	-2															-2.0	-2.0	Unsafe	Unsafe		
Water (control) - O/N from 100°C to RT	-2	-2	-2	-2	-2	-2	-2	-2	-2	-2	-2	-2	-2	-2	-2	-2	-2	-2	-2	-2	-2	-2	-2.0	-2.0	Unsafe	Unsafe	Unsafe	Unsafe
Water (control) - unheated, unmixed (2)	2	2	1	2	0	1	0	1	2	2	1	2	2	2	0	-2	0	1	0	1	0	2	1.2	0.6	Safe	Safe	Safe	Safe
Water (control) - ether extracted (2)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.0	0.0	Safe	Safe	Safe	Safe
Water (control) - 48H @ 37°C (2)	0	0	0	0	0	0	0	-1	0	0	0	0	0	2	0	0	0	2	0	0	0	-2	0.3	-0.4	Safe	Safe	Safe	Unsafe
Water (control) - 24H @ 80°C (2)	-2	-2	-2	-2	-2	-2	-2	-2		-2		-2		-2		-2		-2					-2.0	-2.0	Unsafe	Unsafe		
Water (control) - 40H @ 80°C (2)	-2	-2	-2	-2	-2	-2	-2	-2															-2.0	-2.0	Unsafe	Unsafe		
Water (control) - O/N from 100°C to RT (2)	-2	-2	-2	-2	-2	-2	-2	-2	-2	-2	-2	-2	-2	-2	-2	-2	-2	-2	-2	-2	-2	-2	-2.0	-2.0	Unsafe	Unsafe	Unsafe	Unsafe
Acetic acid	-2	-2	-2	-2	-2	-2	-2	-2	-2	-2	-2	-2	-2	-2	-2	-2	-1	-1	-2	-2	-2	-2	-1.9	-2.0	Unsafe	Unsafe	Unsafe	Unsafe
Acetone	0	-1	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	-0.1	0.5	Safe	Safe	Safe	Safe
1:1 acetone:IMS	0	-1	0	-1	1	1	1	1	0	0	0	0	0	1	0	0	0	-1	1	1	1	1	-0.1	1.0	Safe	Safe	Safe	Safe
Acrylic emulsion	0	-1	0	-2	2	1	2	2	0	0	0	-1	-1	-2	0	-2	-1	-1	1	1	2	2	-0.8	1.6	Unsafe	Safe	Unsafe	Safe
Alum																												
Alum (2)																												
Alum column cleaned																												
Alum re-ppt																												
Ammonium hydroxide	-1	-2	-2	-2	0	0	0	0	-1	-2	-1	-1	0	-1	-1	-2	-1	0	0	0	0	0	-1.2	0.0	Unsafe	Safe	Unsafe	Safe
Amyl acetate	0	-1	0	-1	-1	0	0	0	0	0	0	0	0	0	1	1	0	1	0	0	0	0	0.1	-0.1	Safe	Safe	Safe	Safe
Arsenic trioxide	0	-1	-1	-1	0	0	0	-1	-1	-1	-1	-1	0	0	0	1	0	1	0	1	0	0	-0.4	0.0	Safe	Safe	Safe	Safe
Benzene	-1	-1	-1	-1	-2	-2	-2	-2	-1	-1	-1	-1	-1	-1	0	0	-1	0	-2	-1	-2	-1	-0.8	-1.8	Unsafe	Unsafe	Unsafe	Unsafe
Carbon tetrachloride	-1	-2	-1	-2	-2	-2	-2	-2	-1	-1	-1	-1	-1	-1	0	0	-1	0	-1	-1	-1	-1	-0.9	-1.5	Unsafe	Unsafe	Unsafe	Unsafe
Cellulose nitrate	0	-1	0	-1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	-0.1	0.0	Safe	Safe	Unsafe	Safe
Chloroform	0	-1	0	-1	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	-0.1	0.0	Safe	Safe	Safe	Safe
Detergent	-2	-2	-2	-2	-1	-1	2	0	-2	-2	-2	-2	-2	-2	-2	-2	-2	-2	-1	-1	2	2	-2.0	0.3	Unsafe	Safe	Unsafe	Safe

Treatment	Scatterplot observations								Boxplot observations														Effect score		Plot conclusion		Mann-Whitney conclusion	
	2 = all samples ≥ control standard deviation; 1 = all samples ≥ control mean, 0 = samples above and below control mean, -1 = all samples ≤ control mean, -2 = all samples ≤ control standard deviation								*2 = treated samples above maximum value of water control, 1 = treated samples above median of water control, 0 = treated samples included median of water control, -1 = treated samples below median of water control, -2 = treated samples below minimum value of water control)*														*TY ave = mean of all TY scores; SM ave = mean of all SM scores*		*Safe = x ≥ -0.4; Unsafe = x < -0.4*		*Unsafe = small U value, and at least one p < .05, and median of treatment less than control; otherwise, safe*	
	TYA1	TYA2	TYB1	TYB2	SMA1	SMA2	SMB1	SMB2	TYA1	TYA2	TYB1	TYB2	TYC1	TYC2	TYD1	TYD2	TYE1	TYE2	SMA1	SMA2	SMB1	SMB2	TY ave	SM ave	TY	SM	TY	SM
Detergent (2)	-2	-2	-2	-2	-2	1	-2	2	-2	-2	-2	-2	-2	-1	-2	2	-2		-2	1		2	-1.6	0.0	Unsafe	Safe	Unsafe	
Detergent re-ppt		-2		-2		-1		0												-1		2	-2.0	0.0	Unsafe	Safe		
Detergent - 40H @ 80°C	-2	-2	-2	-2	-2	-2	-2	-2															-2.0	-2.0	Unsafe	Unsafe		
Detergent - 40H @ 80°C (2)	-2	-2	-2	-2	-2	-2	-2	-2															-2.0	-2.0	Unsafe	Unsafe		
EDTA	0	0	-2	-2	-2	-1	0	0	0	0	-2	-2	-2	-2	-2	-2	-2	-2	0	-1	1	2	-1.4	-0.1	Unsafe	Safe	Unsafe	Safe
EDTA (2)	-2	-1	-2	-2	-1	0	-2	0	-2	0	-2	-2	-2	2	-2	2	-2		-1	2		2	-1.2	0.0	Unsafe	Safe		
EDTA re-ppt	-2	-1		-2	2	1		2	-2	1		-2		-2		-2		-2	2	1		2	-1.6	1.7	Unsafe	Safe		
Ethanol	-1	-2	-1	-2	1	1	1	0	-1	-1	-1	-1	0	0	0	-1	-1	-1	1	1	1	1	-0.9	0.9	Unsafe	Safe	Unsafe	Safe
1:1 ethanol:ether	-1	-1	-1	-1	0	0	0	0	-1	0	0	0	0	0	0	1	0	0	0	0	0	0	-0.3	0.0	Safe	Safe	Safe	Safe
Ethyl acetate	-1	-1	-1	-1	-1	0	0	0	-1	-1	-1	-1	0	0	1	1	0	0	0	0	0	0	-0.4	-0.1	Safe	Safe	Safe	Safe
Gasoline	-2	-2	-2	-2	-2	-2	-2	-2	-2	-2	-2	-2	-2	-2	-1	-1	-1	-1	-2	-2	-2	-2	-1.7	-2.0	Unsafe	Unsafe	Unsafe	Unsafe
Gum arabic	-2	-2	-2	-2	-1	0	0	-2	-2	-2	-2	-2	-2	-2	-2	-2	-2	-2	-1	1	2		-2.0	-0.1	Unsafe	Safe	Unsafe	
Gum arabic re-ppt	-1	-2	-2	-2	-2	0	0	-2	-1	-2	-2	-2	-2	-2	-2	-2	-2	-2		0	2		-1.9	-0.3	Unsafe	Safe	Unsafe	Safe
Hydrogen peroxide	-2	-2	-2	-2	-2	-2	-2	-2	-2	-2	-2	-2	0	-1	-2	-2	-1	-1	-2	-2	-2	-2	-1.6	-2.0	Unsafe	Unsafe	Unsafe	Unsafe
IMS	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	1	0	0	0	0	0	0	0.3	0.0	Safe	Safe	Safe	Safe
Kerosene	-1	-2	-1	-2	-1	-1	-1	-1	-1	-1	-1	-1	-1	0	0	1	-1	0	-1	-1	0	-1	-0.8	-0.9	Unsafe	Unsafe	Unsafe	Unsafe
Linseed oil	-1	-2	-1	-2	-2	-2	-2	-2	-1	-1	-1	-1	-1	-1	0	-1	0	0	-2	-2	-2	-2	-0.9	-2.0	Unsafe	Unsafe	Unsafe	Unsafe
Mercury (II) chloride	-2	0	-2	-2	-2	-2	-2	-2	-2	0	-2	-1	-2	-2	-2	-2	-2	-2	-2	-2	-2		-1.6	-2.0	Unsafe	Unsafe	Unsafe	
Mercury (II) chloride (2)	-2	-2	-2	-2	-2	-2	-2	-2	-2	-2	-2	-2	-2	-2	-2	-2	-2	-2	-2	-2	-2	-2	-2.0	-2.0	Unsafe	Unsafe	Unsafe	Unsafe
Mercury (II) chloride re-ppt																												
Methylmethacrylate/ethylacrylate	0	-1	0	-1	0	0	0	0	0	0	0	0	0	0	0	0	-1	-1	0	0	0	0	-0.3	0.0	Safe	Safe	Unsafe	Safe
Mineral oil	-2	-2	-2	-2	-2	-2	-2	-2	-2	-2	-2	-2	-2	-2	-2	-2	-2	-2	-2	-2	-2	-2	-2.0	-2.0	Unsafe	Unsafe	Unsafe	Unsafe
Oxalic acid																												
Oxalic acid (2)																												
Oxalic acid column cleaned																												
Oxalic acid re-ppt																												
Pepsin	-2	-2	-2	-2	-2	-2	-2	-2	-2	-2	-2	-2	-2	-2	-2	-2	-2	-2	-2	-2	-2	-2	-2.0	-2.0	Unsafe	Unsafe	Unsafe	Unsafe

Treatment	Scatterplot observations								Boxplot observations														Effect score		Plot conclusion		Mann-Whitney conclusion	
	2 = all samples ≥ control standard deviation; 1 = all samples ≥ control mean, 0 = samples above and below control mean, -1 = all samples ≤ control mean, -2 = all samples ≤ control standard deviation								*2 = treated samples above maximum value of water control, 1 = treated samples above median of water control, 0 = treated samples included median of water control, -1 = treated samples below median of water control, -2 = treated samples below minimum value of water control)*														*TY ave = mean of all TY scores; SM ave = mean of all SM scores*		*Safe = x ≥ -0.4; Unsafe = x < -0.4*		*Unsafe = small U value, and at least one p < .05, and median of treatment less than control; otherwise, safe*	
	TYA1	TYA2	TYB1	TYB2	SMA1	SMA2	SMB1	SMB2	TYA1	TYA2	TYB1	TYB2	TYC1	TYC2	TYD1	TYD2	TYE1	TYE2	SMA1	SMA2	SMB1	SMB2	TY ave	SM ave	TY	SM	TY	SM
Pepsin (2)	-2	-2	-2	-2	-2	-2	-2	-2	-2	-2	-2	-2	-2	-2	-2	-2	-2	-2	-2	-2	-2	-2	-2.0	-2.0	Unsafe	Unsafe	Unsafe	Unsafe
Pepsin - 48H @ 37°C	-2	-2	-2	-2	-2	-2	-2	-2	-2														-2.0	-2.0	Unsafe	Unsafe	Unsafe	
Pepsin - 48H @ 37°C (2)	-2	-2	-2	-2	-2	-2	-2	0	-2	-2	-2	-2	-2	-2	-2	-2	-2	-2	-2	-2	-2	1	-2.0	-1.4	Unsafe	Unsafe	Unsafe	
Poly(vinyl) butyral resin	-1	-2	-1	-2	0	0	0	0	-1	-1	-1	-1	-1	-1	0	0	-1	-1	0	0	1	1	-1.0	0.3	Unsafe	Safe	Unsafe	Safe
Potassium carbonate	0	0	-1	-1	2	2	2	2	0	0	-1	-1	-2	-2	-2	-2	-2	-2	2	2	2	2	-1.1	2.0	Unsafe	Safe	Unsafe	Safe
Potassium carbonate - 6H @ 80°C	-2	-2	-2	-2	-2	-2	-2	-2	-2	-2	-2	-2	-2	-2	-2	-2	-2	-2	-2	-2	-2	-2	-2.0	-2.0	Unsafe	Unsafe	Unsafe	Unsafe
PVAC	-1	-2	-2	-2	0	0	1	0	-1	-1	-1	-1	-2	-1	0	0	-1	0	0	0	1	1	-1.1	0.4	Unsafe	Safe	Unsafe	Safe
PVAC/PVAL	-2	-2	-2	-2	-2	0	0	-1	-2	-2	-2	-2	-2	-2	-2	-2	-1	-1	-1	-1	0	-1	-1.9	-0.8	Unsafe	Unsafe	Unsafe	Unsafe
Shellac	-1	-2	-2	-2	1	0	2	1	-1	-2	-2	-2	-2	-2	0	-2	0	-2	1	0	2	1	-1.6	1.0	Unsafe	Safe	Unsafe	Safe
Sodium bicarbonate	-2	-2	-2	-2	0	0	0	2	-1	-2	-2	-2	-2	-2	-2		-2	-2	0	1	2	2	-1.9	0.9	Unsafe	Safe	Unsafe	Safe
Sodium bicarbonate (2)	-2	-2	-2	-2	-2	-2	-2	-2	-2		-2				-1		-2						-1.9	-2.0	Unsafe	Unsafe		
Sodium bicarbonate re-ppt	-2	-2	-2	-2	-1	0	1	1	-2	-2	-2	-2	-2	-2	-2	-2	-2	-2	-1	0	1	1	-2.0	0.3	Unsafe	Safe	Unsafe	Safe
Sodium bicarbonate - 24H @ 80°C	-2	-2	-2	-2	-2	-2	-2	-2	-2	-2	-2	-2	-2	-2	-2	-2	-1	-2	-2	-2	-2	-2	-1.9	-2.0	Unsafe	Unsafe	Unsafe	Unsafe
Sodium bicarbonate - 24H @ 80°C (2)	-2	-1	-2	-2	-2	-2	-2	-2	-2	-2	-2	-2	-2	-2	-2	-2	-2	-2	-2	-2	-2	-2	-1.9	-2.0	Unsafe	Unsafe	Unsafe	
Sodium carbonate	-2	-2	-2	-2	1	1	2	1	-2	-2	-2	-2	0	-1	-2	-2	-2	-2	1	1	1	1	-1.8	1.1	Unsafe	Safe	Unsafe	Safe
Sodium chloride	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	1	0	1	0	1	0	1	0.1	0.4	Safe	Safe	Safe	Safe
Sodium chloride - 24H @ 37°C	0	0	0	0	0	0	0	1	0	0	0	0	0	-2	1	-2	0	-2	0	0	0	1	-0.4	0.3	Safe	Safe	Unsafe	Safe
Sodium hydroxide	-2	-2	-2	-2	-2	-2	-2	-2	-1	-2	-2	-2	-2	-2	0	-1	0	0	-2	-2	-2	-2	-1.4	-2.0	Unsafe	Unsafe	Unsafe	Unsafe
Sodium hypochlorite																												
Sodium hypochlorite (2)																												
Sodium hypochlorite re-ppt																												
Sodium perborate																												
Sodium perborate (2)																												
Sodium perborate-O/N from 100°C to RT																												
Sodium perborate-O/N from 100°C to RT (2)																												
Sodium perborate re-ppt	-2	-2	-2	-2	0	-2	-2	-2	-2		-2		-2		-2		-2		1				-2.0	-1.0	Unsafe	Unsafe		
Sodium perborate column cleaned	-2	-2	-2	-2	-2	-2	0	-1	-2	-2	-2	-2							-2	-2	1	-1	-2.0	-1.1	Unsafe	Unsafe		

Treatment	Scatterplot observations								Boxplot observations														Effect score		Plot conclusion		Mann-Whitney conclusion	
	2 = all samples ≥ control standard deviation; 1 = all samples ≥ control mean, 0 = samples above and below control mean, -1 = all samples ≤ control mean, -2 = all samples ≤ control standard deviation								*2 = treated samples above maximum value of water control, 1 = treated samples above median of water control, 0 = treated samples included median of water control, -1 = treated samples below median of water control, -2 = treated samples below minimum value of water control)*														*TY ave = mean of all TY scores; SM ave = mean of all SM scores*		*Safe = x ≥ -0.4; Unsafe = x < -0.4*		*Unsafe = small U value, and at least one p < .05, and median of treatment less than control; otherwise, safe*	
	TYA1	TYA2	TYB1	TYB2	SMA1	SMA2	SMB1	SMB2	TYA1	TYA2	TYB1	TYB2	TYC1	TYC2	TYD1	TYD2	TYE1	TYE2	SMA1	SMA2	SMB1	SMB2	TY ave	SM ave	TY	SM	TY	SM
Sodium sulfide	-2	-2	-2	-2	-2	-2	-2	-2	-2	-2	-2	-2	-2	-2	-2	-2	-2	-2	-2	-2	-2	-2	-2.0	-2.0	Unsafe	Unsafe	Unsafe	Unsafe
Sodium sulfide - 6H @ 80°C	-2	-2	-2	-2	-2	-2	-2	-2	-2	-2	-2	-2	-2	-2	-2	-2	-2	-2	-2	-2	-2	-2	-2.0	-2.0	Unsafe	Unsafe	Unsafe	Unsafe
Toluene	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0.1	0.0	Safe	Safe	Safe	Safe
Trichloroethylene	0	0	0	0	0	0	1	0	0	0	0	0	1	1	1	1	0	0	0	0	1	1	0.3	0.4	Safe	Safe	Safe	Safe
Turpentine	-2	-2	-2	-2	-2	-2	-2	-2	-2	-2	-2	-2	-2	-2	-2	-2	-2	-2			-2		-2.0	-2.0	Unsafe	Unsafe	Unsafe	
White spirit	-1	-2	-1	-2	-2	-1	0	-2	-1	-1	-1	-1	-1	0	0	0	0	0	-1	-1	0	-1	-0.8	-1.0	Unsafe	Unsafe	Unsafe	Unsafe
Xylene	0	-1	0	-1	0	0	0	-1	0	0	0	0	0	1	0	1	0	0	0	0	0	0	0.0	-0.1	Safe	Safe	Safe	Safe

Table 12. Summary table of scatterplot and boxplot scores, with effect score summary, and damage determination based on combined plot data and Mann-Whitney data. "H" = hours, "O/N" = overnight and "RT" = room temperature.

5.1.6. Quantification of DNA preservation

DNA preservation was quantified using the peak height ratio data. Peak height ratio means were calculated for each set of treatment samples and control samples. Using the water control peak height ratio as a baseline (theoretically, 100% DNA preservation, although it is acknowledged that some damage would result from exposure to water, this effect was equal across all samples), both positive and negative preservation effects due to conservation chemical treatment could be estimated. Treated sample means were divided by the control sample means (repeated treatment sample means were divided by the repeated water control mean), and the value obtained expressed as a percentage. To assess any differences in preservation between the two different fragments and between runs, the average and standard deviation of the values for TY1, TY2, SM1 and SM2 groups were calculated. Averages and standard deviations were also calculated for all TY and SM data. Finally, the overall DNA percent preservation was calculated, represented by the average and standard deviation of all calculated values for each treatment. All DNA preservation percentages calculated are presented in Table 13.

Assessing the effects of treatments on DNA using the percentage of DNA preserved calculation allowed sample variation to be taken into account better than the methods previously described. Although both scatterplot and boxplot observations noted whether treated sample ratios ranged both above and below the control mean or median, respectively, how far above or below the mean or median was not accounted for, as these methods were not quantitative. Additionally, due to the small sample size of each of the treatment groups, the Mann-Whitney test could not detect a significant difference between the ratios of some treatments and the control samples.

The ratio variation for each of the peaks within the control groups was explored to assess the level of variation that might be acceptable within the treatment groups. The average and standard deviation was calculated for each run for the TY and SM peak height ratios. It was found that the average standard deviation for the TY ratio was 11.5% and 12.1% for run 1 and run 2, respectively, and the average standard deviation for the SM ratio was 9.8% and 10.9% for run 1 and run 2, respectively. When using the percentage DNA preservation data to identify safe treatments, it was decided that standard deviations exceeding approximately 12% indicated too much variation in sample data to be reliable. (It was also noted that the TYC peaks had the highest variance, 39.8% for run 1 and 42.1% for run 2. It is therefore recommended to use multiple sequences as well as sequences that give multiple peaks for this method of assessing the effects of treatments on DNA, so that peaks prone to extreme variation can be identified and the results adjusted.)

Percentage DNA preservation data was used in conjunction with the other assessment methods to determine which treatments tested are safe for DNA preservation.

DNA preservation (all values as %)																												
Treatment	**TYA1**	**TYB1**	**TYC1**	**TYD1**	**TYE1**	***TY1 ave***	***TY1 st dev***	**TYA2**	**TYB2**	**TYC2**	**TYD2**	**TYE2**	***TY2 ave***	***TY2 st dev***	**Total TY ave**	**Total TY st dev**	**SMA1**	**SMB1**	***SM1 ave***	***SM1 st dev***	**SMA2**	**SMB2**	***SM2 ave***	***SM2 st dev***	**Total SM ave**	**Total SM st dev**	**Overall ave**	**Overall st dev**
Water (control)	100.0	100.0	100.0	100.0	100.0	*100.0*	*0.0*	100.0	100.0	100.0	100.0	100.0	*100.0*	*0.0*	**100.0**	**0.0**	100.0	99.9	*100.0*	*0.1*	100.0	100.0	*100.0*	*0.0*	**100.0**	**0.1**	**100.0**	**0.0**
Water (control) - 48H @ 37°C	81.1	79.2	73.5	96.8	92.1	*84.5*	*9.6*	75.6	72.9	33.5	52.9	41.1	*55.2*	*18.7*	**69.9**	**20.9**	68.1	72.4	*70.3*	*3.0*	75.1	89.6	*82.4*	*10.3*	**76.3**	**9.3**	**71.7**	**18.2**
Water (control) - 6H @ 80°C	2.6	2.6	1.3	1.7	1.1	*1.9*	*0.7*								**1.9**	**0.7**											**1.9**	**0.7**
Water (control) - 40H @ 80°C																												
Water (control) - O/N from 100°C to RT	52.1	51.1	51.0	55.8	48.3	*51.6*	*2.7*	55.4	54.5	25.5	34.3	26.9	*39.3*	*14.7*	**45.5**	**11.9**	21.1	12.3	*16.7*	*6.2*	30.7	35.1	*32.9*	*3.1*	**24.8**	**10.2**	**39.6**	**14.7**
Water (control) (2)	100.0	100.0	100.0	100.0	100.0	*100.0*	*0.0*	100.0	100.0	100.0	100.0	100.0	*100.0*	*0.0*	**100.0**	**0.0**	100.0	99.9	*100.0*	*0.1*	100.0	99.9	*100.0*	*0.1*	**100.0**	**0.1**	**100.0**	**0.0**
Water (control) - unheated, unmixed (2)	133.7	104.8	118.9	99.4	90.5	*109.5*	*17.0*	115.1	115.7	113.3	91.9	111.2	*109.4*	*10.0*	**109.4**	**13.1**	111.8	116.3	*114.1*	*3.2*	119.8	120.8	*120.3*	*0.7*	**117.2**	**4.1**	**111.7**	**11.7**
Water (control) - ether extracted (2)	107.2	103.9	108.8	104.3	101.5	*105.1*	*2.9*	103.4	102.3	106.5	102.0	101.5	*103.1*	*2.0*	**104.1**	**2.6**	105.7	106.3	*106.0*	*0.5*	102.6	105.2	*103.9*	*1.9*	**104.9**	**1.6**	**104.4**	**2.3**
Water (control) - 48H @ 37°C (2)	96.6	94.3	96.3	95.6	94.9	*95.6*	*0.9*	95.4	96.4	160.5	114.3	201.7	*133.7*	*46.3*	**114.6**	**36.8**	95.8	95.5	*95.6*	*0.2*	90.8	71.9	*81.4*	*13.4*	**88.5**	**11.3**	**107.2**	**33.4**
Water (control) - 24H @ 80°C (2)																												
Water (control) - 40H @ 80°C (2)																												
Water (control) - O/N from 100°C to RT (2)	81.7	79.3	83.7	72.6	69.9	*77.4*	*5.9*	72.4	70.9	76.4	67.3	65.0	*70.4*	*4.4*	**73.9**	**6.2**	47.8	47.2	*47.5*	*0.4*	45.1	44.9	*45.0*	*0.1*	**46.2**	**1.5**	**66.0**	**14.0**
1:1 acetone:IMS	96.7	91.6	89.0	104.0	93.5	*95.0*	*5.8*	86.6	86.2	74.6	96.1	99.4	*88.6*	*9.7*	**91.8**	**8.3**	104.2	109.4	*106.8*	*3.7*	109.2	103.6	*106.4*	*4.0*	**106.6**	**3.1**	**96.0**	**9.9**
1:1 ethanol:ether	91.8	91.8	86.7	104.8	98.3	*94.7*	*7.0*	86.4	85.3	70.0	97.0	109.9	*89.7*	*14.8*	**92.2**	**11.2**	104.3	106.6	*105.4*	*1.6*	104.8	100.0	*102.4*	*3.3*	**103.9**	**2.8**	**95.5**	**10.9**
Acetic acid	48.1	48.4	39.1	49.2	53.8	*47.7*	*5.4*	43.6	44.5	36.1	51.8	73.4	*49.9*	*14.3*	**48.8**	**10.2**	23.0	16.9	*20.0*	*4.3*	20.8	9.0	*14.9*	*8.3*	**17.4**	**6.2**	**39.8**	**17.2**
Acetone	100.4	98.2	91.3	107.4	97.3	*98.9*	*5.8*	91.4	90.9	75.0	97.8	103.5	*91.7*	*10.7*	**95.3**	**8.9**	100.1	107.7	*103.9*	*5.4*	101.5	102.0	*101.7*	*0.3*	**102.8**	**3.4**	**97.5**	**8.4**
Acrylic emulsion	95.7	89.9	58.2	80.8	56.6	*76.3*	*18.0*	89.1	80.8	43.8	69.5	58.2	*68.3*	*18.0*	**72.3**	**17.5**	124.5	160.6	*142.5*	*25.5*	134.1	162.2	*148.1*	*19.9*	**145.3**	**18.9**	**93.1**	**38.3**
Alum																												
Alum (2)																												
Ammonium hydroxide	82.8	80.6	72.7	77.4	71.1	*76.9*	*5.0*	72.5	73.6	56.6	72.4	91.3	*73.3*	*12.3*	**75.1**	**9.0**	86.9	94.2	*90.5*	*5.2*	90.9	91.1	*91.0*	*0.2*	**90.8**	**3.0**	**79.6**	**10.6**
Amyl acetate	100.5	98.4	93.4	113.2	99.8	*101.1*	*7.3*	92.4	91.4	77.5	103.4	110.7	*95.1*	*12.7*	**98.1**	**10.3**	94.3	101.1	*97.7*	*4.8*	95.4	96.2	*95.8*	*0.6*	**96.7**	**3.0**	**97.7**	**8.7**
Arsenic trioxide	94.5	94.4	83.4	105.5	105.4	*96.7*	*9.2*	87.3	86.1	72.3	100.9	119.0	*93.1*	*17.7*	**94.9**	**13.4**	99.4	102.5	*100.9*	*2.2*	100.9	94.6	*97.8*	*4.5*	**99.3**	**3.4**	**96.2**	**11.5**
Benzene	85.7	85.0	76.7	94.4	86.8	*85.7*	*6.3*	79.8	79.4	64.3	89.8	94.6	*81.6*	*11.7*	**83.7**	**9.1**	76.7	77.5	*77.1*	*0.6*	77.9	73.2	*75.5*	*3.4*	**76.3**	**2.2**	**81.6**	**8.4**
Carbon tetrachloride	86.8	85.0	74.6	95.1	89.5	*86.2*	*7.5*	81.4	81.7	64.7	93.1	104.2	*85.0*	*14.7*	**85.6**	**11.1**	81.2	83.8	*82.5*	*1.8*	82.3	77.5	*79.9*	*3.4*	**81.2**	**2.7**	**84.4**	**9.5**
Cellulose nitrate	85.4	86.0	80.2	101.2	93.1	*89.2*	*8.1*	75.9	76.2	60.0	88.3	94.1	*78.9*	*13.1*	**84.0**	**11.6**	99.1	101.8	*100.5*	*1.9*	101.7	97.5	*99.6*	*3.0*	**100.0**	**2.1**	**88.6**	**12.3**
Chloroform	95.4	94.0	89.9	109.1	101.1	*97.9*	*7.4*	88.8	88.2	76.7	106.5	109.0	*93.8*	*13.6*	**95.9**	**10.6**	97.1	102.4	*99.7*	*3.8*	96.6	98.1	*97.4*	*1.1*	**98.5**	**2.7**	**96.6**	**9.0**
Detergent	55.7	41.3	33.4	44.7	24.9	*40.0*	*11.6*	48.5	38.5	27.5	36.7	26.2	*35.5*	*9.0*	**37.7**	**10.1**	90.2	165.6	*127.9*	*53.3*	91.6	175.8	*133.7*	*59.5*	**130.8**	**46.3**	**64.3**	**49.7**
Detergent - 40H @ 80°C																												
Detergent - 40H @ 80°C (2)																												
Detergent (2)	72.3	58.2	79.7	77.6	63.1	*70.2*	*9.3*	62.6	45.9	92.9	109.1		*77.6*	*28.6*	**73.5**	**19.1**	85.1		*85.1*		109.7	177.3	*143.5*	*47.8*	**124.0**	**47.7**	**86.1**	**34.7**
Detergent re-ppt																					94.6	191.6	*143.1*	*68.6*	**143.1**	**68.6**	**143.1**	**68.6**

DNA preservation (all values as %)																										
Treatment	**TYA1**	**TYB1**	**TYC1**	**TYD1**	**TYE1**	***TY1 ave***	***TY1 st dev***	**TYA2**	**TYB2**	**TYC2**	**TYD2**	**TYE2**	***TY2 ave***	***TY2 st dev***	**Total TY ave**	**Total TY st dev**	**SMA1**	**SMB1**	***SM1 ave***	***SM1 st dev***	**SMA2**	**SMB2**	***SM2 ave***	***SM2 st dev***	**Total SM ave**	**Total SM st dev**	**Overall ave**	**Overall st dev**
EDTA	85.8	58.2	36.6	37.1	25.5	*48.6*	*23.9*	90.5	58.6	34.1	47.5	34.1	*53.0*	*23.4*	**50.8**	**22.4**	72.7	178.9	*125.8*	*75.1*	88.1	144.9	*116.5*	*40.2*	**121.2**	**49.5**	**70.9**	**44.7**
EDTA (2)	80.6	66.8	76.3	82.9	68.0	*74.9*	*7.3*	99.3	74.9	224.8	132.3		*132.8*	*65.7*	**100.6**	**50.7**	98.7		*98.7*		135.8	145.7	*140.7*	*7.1*	**126.7**	**24.8**	**107.2**	**46.1**
EDTA re-ppt	66.9					*66.9*		110.7	71.5	33.8	52.7	40.4	*61.8*	*30.8*	**62.7**	**27.7**	125.2		*125.2*		110.4	196.3	*153.3*	*60.7*	**143.9**	**45.9**	**89.8**	**51.5**
Ethanol	88.5	87.3	84.8	95.8	88.2	*88.9*	*4.1*	80.0	79.1	68.0	90.2	98.3	*83.1*	*11.6*	**86.0**	**8.7**	103.3	108.2	*105.7*	*3.5*	108.3	104.0	*106.1*	*3.1*	**105.9**	**2.7**	**91.7**	**11.9**
Ethyl acetate	93.2	92.5	84.5	108.4	101.6	*96.0*	*9.2*	86.3	86.2	70.5	98.1	109.2	*90.1*	*14.5*	**93.1**	**11.9**	95.8	99.5	*97.7*	*2.6*	98.7	96.3	*97.5*	*1.7*	**97.6**	**1.8**	**94.4**	**10.2**
Gasoline	77.2	75.5	69.3	88.8	82.6	*78.7*	*7.4*	71.2	70.5	57.3	83.4	90.4	*74.6*	*12.8*	**76.6**	**10.1**	70.9	75.4	*73.2*	*3.2*	71.6	70.2	*70.9*	*1.0*	**72.0**	**2.3**	**75.3**	**8.7**
Gum arabic	78.0	57.1	47.8	60.9	36.0	*56.0*	*15.6*	68.3	53.0	30.1	47.7	31.9	*46.2*	*15.8*	**51.1**	**15.7**					107.0		*107.0*		**107.0**		**56.2**	**22.5**
Gum arabic re-ppt	80.5	73.2	41.9	52.4	29.0	*55.4*	*21.4*	67.2	50.4	32.4	52.7	32.6	*47.1*	*14.8*	**51.2**	**17.9**		224.5	*224.5*		100.9		*100.9*		**162.7**	**87.4**	**69.8**	**53.3**
Hydrogen peroxide	68.3	69.6	72.1	75.5	75.0	*72.1*	*3.2*	61.7	63.2	57.9	69.0	76.9	*65.7*	*7.4*	**68.9**	**6.3**	54.5	38.4	*46.4*	*11.3*	53.8	36.4	*45.1*	*12.3*	**45.8**	**9.7**	**62.3**	**12.9**
IMS	102.0	100.9	96.2	115.1	102.0	*103.2*	*7.0*	96.6	95.4	81.5	109.3	113.0	*99.2*	*12.5*	**101.2**	**9.8**	96.5	103.6	*100.1*	*5.0*	96.9	96.5	*96.7*	*0.3*	**98.4**	**3.5**	**100.4**	**8.4**
Kerosene	87.1	86.3	79.9	100.4	94.4	*89.6*	*7.9*	84.3	82.4	66.9	94.9	104.1	*86.5*	*14.0*	**88.1**	**10.9**	90.7	93.4	*92.1*	*2.0*	89.0	86.9	*87.9*	*1.5*	**90.0**	**2.8**	**88.6**	**9.2**
Linseed oil	79.7	80.8	71.4	92.5	107.8	*86.4*	*14.1*	74.3	74.7	58.5	85.0	116.0	*81.7*	*21.4*	**84.1**	**17.3**	63.2	54.9	*59.1*	*5.9*	62.1	53.3	*57.7*	*6.2*	**58.4**	**5.0**	**76.7**	**18.9**
Mercury (II) chloride	44.4	27.7	22.7	27.8	14.0	*27.3*	*11.1*	68.2	53.6	18.0	19.1	19.3	*35.6*	*23.7*	**31.5**	**18.0**					30.7		*30.7*		**30.7**		**31.4**	**17.0**
Mercury (II) chloride (2)	47.3	44.7	43.6	43.7	43.2	*44.5*	*1.7*	46.7	45.4	42.2	42.2	41.8	*43.6*	*2.2*	**44.1**	**1.9**	34.8	40.4	*37.6*	*4.0*	34.7	40.8	*37.8*	*4.4*	**37.7**	**3.4**	**42.2**	**3.8**
Mercury (II) chloride re-ppt																												
Methylmethacrylate/ethylacrylate	92.8	92.9	87.9	96.9	82.0	*90.5*	*5.7*	83.9	83.9	70.8	87.8	87.7	*82.8*	*7.0*	**86.7**	**7.3**	90.2	93.0	*91.6*	*1.9*	92.8	88.5	*90.7*	*3.1*	**91.1**	**2.2**	**87.9**	**6.5**
Mineral oil	26.5	25.9	20.0	24.3	21.4	*23.6*	*2.9*	27.4	27.4	21.3	29.7	30.3	*27.2*	*3.6*	**25.4**	**3.6**	24.9	25.1	*25.0*	*0.1*	23.0	20.3	*21.7*	*1.9*	**23.3**	**2.2**	**24.8**	**3.3**
Oxalic acid																												
Oxalic acid (2)																												
Oxalic acid re-ppt																												
Pepsin	34.9	32.8	19.7	25.4	17.4	*26.0*	*7.7*	32.2	30.0	13.7	20.6	15.0	*22.3*	*8.5*	**24.2**	**7.9**	28.6	41.9	*35.3*	*9.4*	28.3	41.8	*35.0*	*9.6*	**35.1**	**7.7**	**27.3**	**9.1**
Pepsin - 48H @ 37°C	32.6	27.5	16.5	19.0	12.0	*21.5*	*8.4*								**21.5**	**8.4**											**21.5**	**8.4**
Pepsin - 48H @ 37°C (2)	13.4	12.0	13.1	13.0	12.7	*12.8*	*0.5*	14.4	14.4	22.4	16.9	28.5	*19.3*	*6.1*	**16.1**	**5.3**	10.4	17.2	*13.8*	*4.8*	45.1	53.7	*49.4*	*6.1*	**31.6**	**21.1**	**20.5**	**13.2**
Pepsin (2)	45.2	42.9	45.6	44.2	40.6	*43.7*	*2.0*	36.0	38.2	63.5	65.7	79.4	*56.6*	*18.8*	**50.1**	**14.3**	27.5	30.7	*29.1*	*2.3*	34.0	46.9	*40.5*	*9.2*	**34.8**	**8.5**	**45.7**	**14.5**
Poly(vinyl) butyral resin	73.7	71.3	64.1	80.2	65.1	*70.9*	*6.6*	71.3	70.1	52.0	76.3	75.2	*69.0*	*9.9*	**69.9**	**8.0**	98.8	123.7	*111.2*	*17.6*	102.5	117.0	*109.8*	*10.3*	**110.5**	**11.8**	**81.5**	**20.9**
Potassium carbonate	90.1	81.2	43.7	60.8	37.3	*62.6*	*22.9*	93.0	73.5	35.8	54.6	42.6	*59.9*	*23.4*	**61.3**	**21.9**	174.4	234.7	*204.5*	*42.6*	185.9	225.2	*205.6*	*27.8*	**205.0**	**29.4**	**102.3**	**71.2**
Potassium carbonate - 6H @ 80°C	28.8	24.2	12.9	17.9	10.7	*18.9*	*7.6*	35.6	29.3	14.3	22.2	17.6	*23.8*	*8.7*	**21.3**	**8.1**	22.2	46.3	*34.3*	*17.1*	23.3	41.3	*32.3*	*12.8*	**33.3**	**12.4**	**24.8**	**10.6**
PVAC	82.0	80.6	70.9	94.6	89.5	*83.5*	*9.1*	75.9	75.5	58.8	91.8	101.4	*80.7*	*16.4*	**82.1**	**12.6**	101.7	109.6	*105.6*	*5.6*	101.9	99.3	*100.6*	*1.9*	**103.1**	**4.5**	**88.1**	**14.6**
PVAC/PVAL	66.8	64.0	58.3	77.5	78.0	*68.9*	*8.6*	65.4	64.8	51.8	78.3	87.1	*69.5*	*13.6*	**69.2**	**10.7**	87.8	95.9	*91.8*	*5.8*	94.0	90.2	*92.1*	*2.7*	**92.0**	**3.7**	**75.7**	**14.0**
Shellac	85.0	74.2	58.6	82.1	74.9	*75.0*	*10.3*	70.9	72.2	33.2	50.3	40.2	*53.4*	*17.7*	**64.2**	**17.8**	120.0	157.3	*138.6*	*26.4*	105.9	122.3	*114.1*	*11.7*	**126.4**	**21.9**	**81.9**	**34.3**
Sodium bicarbonate	67.4	54.0	41.6	57.5	28.9	*49.9*	*14.9*	62.7	48.2	35.7		32.3	*44.7*	*13.8*	**47.6**	**13.8**	110.6	262.2	*186.4*	*107.2*	103.9	170.5	*137.2*	*47.1*	**161.8**	**73.3**	**82.7**	**66.9**
Sodium bicarbonate - 24H @ 80°C	34.4	32.1	22.6	31.4	30.7	*30.2*	*4.5*	32.3	28.4	13.2	19.3	15.1	*21.7*	*8.3*	**26.0**	**7.8**	15.8	10.0	*12.9*	*4.1*	34.3	31.5	*32.9*	*2.0*	**22.9**	**11.8**	**25.1**	**8.7**

DNA preservation (all values as %)																												
Treatment	**TYA1**	**TYB1**	**TYC1**	**TYD1**	**TYE1**	***TY1 ave***	***TY1 st dev***	**TYA2**	**TYB2**	**TYC2**	**TYD2**	**TYE2**	***TY2 ave***	***TY2 st dev***	**Total TY ave**	**Total TY st dev**	**SMA1**	**SMB1**	***SM1 ave***	***SM1 st dev***	**SMA2**	**SMB2**	***SM2 ave***	***SM2 st dev***	**Total SM ave**	**Total SM st dev**	**Overall ave**	**Overall st dev**
Sodium bicarbonate - 24H @ 80°C (2)	66.7	57.5	66.7	74.3	79.3	*68.9*	*8.3*	93.6	77.2	73.8	77.0	78.9	*80.1*	*7.8*	**74.5**	**9.6**	30.2	33.8	*32.0*	*2.6*	30.0	32.7	*31.3*	*2.0*	**31.7**	**1.9**	**62.3**	**21.6**
Sodium bicarbonate (2)	85.5	58.7		99.4	65.0	*77.2*	*18.7*								**77.2**	**18.7**											**77.2**	**18.7**
Sodium bicarbonate re-ppt	74.3	61.7	45.5	36.8	27.3	*49.1*	*18.9*	72.7	66.5	38.7	42.2	30.9	*50.2*	*18.3*	**49.7**	**17.6**	84.3	134.2	*109.3*	*35.2*	110.0	132.7	*121.3*	*16.1*	**115.3**	**23.4**	**68.4**	**35.9**
Sodium carbonate	71.0	64.7	65.6	54.3	40.1	*59.1*	*12.3*	64.4	60.3	39.6	42.2	36.4	*48.6*	*12.8*	**53.9**	**13.1**	129.9	146.7	*138.3*	*11.9*	138.7	164.9	*151.8*	*18.5*	**145.1**	**14.9**	**79.9**	**44.7**
Sodium chloride	94.8	94.3	87.1	104.4	99.9	*96.1*	*6.5*	92.0	91.3	76.0	104.2	116.1	*95.9*	*15.1*	**96.0**	**10.9**	109.6	112.5	*111.0*	*2.0*	114.1	109.5	*111.8*	*3.2*	**111.4**	**2.2**	**100.4**	**11.7**
Sodium chloride - 24H @ 37°C	104.9	104.0	97.9	116.1	100.8	*104.7*	*6.9*	99.2	96.9	45.1	65.3	49.8	*71.3*	*25.6*	**88.0**	**25.0**	107.3	113.4	*110.4*	*4.3*	113.3	133.8	*123.5*	*14.6*	**117.0**	**11.6**	**96.3**	**25.4**
Sodium hydroxide	75.8	74.7	70.2	94.2	93.8	*81.7*	*11.4*	69.8	69.2	57.7	87.4	101.1	*77.0*	*17.1*	**79.4**	**13.9**	60.4	57.6	*59.0*	*2.0*	63.8	57.1	*60.4*	*4.7*	**59.7**	**3.1**	**73.8**	**14.9**
Sodium hypochlorite																												
Sodium hypochlorite (2)																												
Sodium hypochlorite re-ppt																												
Sodium perborate																												
Sodium perborate - O/N from 100°C to RT																												
Sodium perborate - O/N from 100°C to RT (2)																												
Sodium perborate (2)																												
Sodium perborate re-ppt	59.4	38.8	35.2	44.2	20.5	*39.6*	*14.1*								**39.6**	**14.1**	101.4										**49.9**	**28.2**
Sodium sulfide	10.1	9.6	4.9	6.9	4.9	*7.3*	*2.5*	9.2	8.5	4.6	7.5	7.3	*7.4*	*1.7*	**7.4**	**2.0**	12.2	16.8	*14.5*	*3.2*	10.2	12.0	*11.1*	*1.3*	**12.8**	**2.8**	**8.9**	**3.3**
Sodium sulfide - 6H @ 80°C	21.2	20.4	11.2	13.7	9.2	*15.1*	*5.4*	18.7	18.1	8.7	12.0	9.4	*13.4*	*4.8*	**14.3**	**4.9**	10.8	14.6	*12.7*	*2.7*	11.1	16.0	*13.5*	*3.5*	**13.1**	**2.6**	**13.9**	**4.3**
Toluene	100.0	99.1	96.0	111.3	103.8	*102.1*	*5.9*	91.9	91.8	78.8	102.2	110.0	*94.9*	*11.8*	**98.5**	**9.6**	99.8	102.5	*101.1*	*1.9*	101.1	98.9	*100.0*	*1.6*	**100.6**	**1.6**	**99.1**	**8.1**
Trichloroethylene	102.3	101.8	93.9	113.0	104.7	*103.1*	*6.8*	94.2	93.4	77.8	106.0	112.5	*96.8*	*13.3*	**100.0**	**10.5**	98.8	105.1	*101.9*	*4.4*	102.2	100.5	*101.3*	*1.2*	**101.6**	**2.7**	**100.4**	**8.9**
Turpentine	11.6	11.7	5.9	7.4	7.3	*8.8*	*2.7*	12.1	12.2	4.6	6.7	5.9	*8.3*	*3.6*	**8.5**	**3.0**		9.6	*9.6*						**9.6**		**8.6**	**2.8**
White spirit	86.5	84.8	69.0	82.9	76.7	*80.6*	*7.2*	81.1	82.3	70.2	95.5	109.5	*87.7*	*15.1*	**83.8**	**11.9**	80.3	86.6	*83.4*	*4.4*	81.6	70.9	*76.3*	*7.6*	**79.8**	**6.5**	**82.7**	**10.5**
Xylene	97.3	95.8	85.6	109.8	102.0	*98.1*	*8.9*	92.7	90.9	71.9	101.6	112.9	*94.0*	*15.1*	**96.0**	**11.9**	97.6	100.2	*98.9*	*1.8*	96.6	94.0	*95.3*	*1.8*	**97.1**	**2.6**	**96.3**	**10.0**

Table 13. Percentages of DNA preserved by treatment and by peak. The average (ave) percentage of DNA preserved and the standard deviation (st dev) were also calculated for both fragments for both runs (TYA1, TYA2, SMA1 and SMA2). "Overall ave" and "st dev" represent the overall average and standard deviation preservation of DNA, which was calculated by averaging the percentages of DNA preserved for all peaks for each treatment. "H" = hours, "O/N" = overnight, "RT" = room temperature, "ave" = average, "st dev" = standard deviation.

5.1.7. Treatments found to be unsafe for DNA in the screening test

The effects of preparation and conservation treatments on DNA *in vitro* were assessed by several methods using peak height ratio data, including a scoring system to assess systematically the data presented in scatterplots and boxplots in combination with statistical analysis using the Mann-Whitney test, as well as the calculation of the percentage of DNA preservation. The results of all methods were largely in agreement with each other, and thus enabled the identification of treatments as either safe or unsafe. For the purposes of this research, "safe" treatments are defined as those treatments that were no more damaging to DNA than suspension for one week in water purified by reverse osmosis, whereas "unsafe" treatments resulted in damage to DNA greater than suspension in deionised water for one week.

In all of the methods used to assess the experimental data, it was observed that the two DNA fragments tested responded to treatment differently. In general, the 112bp TAT fragment was adversely affected by more treatments than the 225bp SRY 4064 fragment. All other factors being equal, it was expected that the longer fragment would incur more apparent damage. Several factors may account for this observed difference. It is known that the bond formed between guanine (G) and cytosine (C) bases is stronger than that between adenine (A) and thymine (T) bases, as the former is composed of 3 hydrogen bonds, whereas the latter is composed of only 2 (Guerra et al. 1999, 2000; Yanson et al. 1979; see also Figure 1), which could result in a more compact conformation with increased resistance to chemical denaturation, as well as a higher resistance to chemical damage generally. In this study, the TAT sequence had a 36% GC content, whereas the SRY 4064 sequence had a 47% GC content (sequences and GC contents are presented in Table 14), which may explain why the TAT fragment was more susceptible to treatment damage than the SRY 4064 fragment. Furthermore, it should be noted that because DNA damage is measured by the ratio between the treated fragment and the standard fragment, the degree of damage calculated using the SM ratio may be overestimated, as the M9 sequence consisted of 32% GC bonds (compared to 47% for SRY 4064), and therefore the M9 fragment may have sustained more damage throughout the clean up and precipitation procedures than the SRY 4064 fragment. This is not likely to be a problem for the TY ratio, however, as both the treated and the standard fragments were of a similar GC composition (36% and 34%, respectively). Other possible explanations for the difference in DNA preservation between the two fragments tested may be due to different bases having specific chemical properties and consequent reactivities, or different sequences having different tertiary conformations, some of which may be more or less protective against chemical attack (Belmont et al. 2001), however investigation of these issues fall outside the scope of this project.

PCR product	PCR product sequence	GC content
YAP- (99bp)	101 AGGACT AGCAATAGCA GGGGAAGATA AAGAAATATA AAAGAAATAT 151 AACATAAGAA GATCAAACCT GTTTTAACTT TAACTTGGTT GGAGTTGGCC 201 CTG	GC% = 34%
TAT (112bp)	1 GAAGGTGCCG TAAAAGTGTG AAATAATCAC CTGAATATTT ACCCTTCTCT 51 CTTGCTGTGC TCTGAAATAT TAAATTAAAA CAACATGAAT TCACAAGTCT 101 ACACTCAGAG TC	GC% = 36%
M9 (214bp)	1 TCAGGACCCT GAAATACAGA ACTGCAAAGA AACGGCCTAA GATGGTTGAA 51 TGCTCTTTAT TTTTCTTTAA TTTAGACATG TTCAAACGTT CAATGTCTTA 101 CATACTTAGT TATGTAAGTA AGGTAGCGCT TACTTCATTA TGCATTTCAA 151 TACTCAAAAA AAATTCCTTT GTGAAATGTT GAAATATTTT TCTAATCTGT 201 TTCACGAGCT TCAA	GC% = 32%
SRY 4064 (225bp)	1 GGTATGACAG GGGATGATGT GATTAATTGA CCTACTGATA AGACTCATTT 51 CAGTAAATGC CACACAAGAA TGTATAATAG GCTGGGTGCT GTGGGTCACA 101 CCTGTAATCC CAGCCCTTCG AGAGGTCAAG GCGAGCGGAT CACAGGGTGA 151 AGAGATTGAG ACCATCCTGG CCAACATGGT GAAACTGGGT CTCTACTAAA 201 AATACAAAAA ATTAGCTGGG CGTGG	GC% = 47%

Table 14. PCR product sequences used in this study and GC content for each sequence.

Conclusions regarding the effects of conservation treatments on DNA *in vitro* based on the scoring system for the scatterplots and boxplots were generally supported by the Mann-Whitney statistic. Repeated treatment samples or additional clean-up samples also considered unsafe were listed with the original treatment rather than being included as a separate treatment. Damaging treatments based on the effect score, Mann-Whitney statistic and electropherogram observations are summarised in Table 15.

Treatments considered unsafe based on TY results	Treatments considered unsafe based on SM results
Acetic acid	Acetic acid
Acrylic emulsion	
Alum (as well as Alum (2), re-ppt and column cleaned presumed damaging)	Alum (as well as Alum (2), re-ppt and column cleaned presumed damaging)
Ammonium hydroxide	
Benzene	Benzene
Carbon tetrachloride	Carbon tetrachloride
Cellulose nitrate [in 1:1 ethanol:ether]	
Detergent - 40H @ 80°C (as well as Detergent - 40H @ 80°C (2))	Detergent - 40H @ 80°C (as well as Detergent - 40H @ 80°C (2))
Detergent (as well as Detergent (2) and re-ppt)	
EDTA (as well as EDTA (2) and re-ppt)	
Ethanol	
Gasoline [in ethanol/turpentine]	Gasoline [in ethanol/turpentine]
Gum arabic (as well as re-ppt)	
Hydrogen peroxide	Hydrogen peroxide
Kerosene	Kerosene
Linseed oil [in turpentine]	Linseed oil [in turpentine]
Mercury (II) chloride (as well as Mercury (II) chloride (2) and re-ppt) [in ethanol]	Mercury (II) chloride (as well as Mercury (II) chloride (2) and re-ppt) [in ethanol]
Methylmethacrylate/ethylacrylate [in acetone]	

Treatments considered unsafe based on TY results	Treatments considered unsafe based on SM results
Mineral oil	Mineral oil
Oxalic acid (as well as Oxalic acid (2), re-ppt and column cleaned presumed damaging)	Oxalic acid (as well as Oxalic acid (2), re-ppt and column cleaned presumed damaging)
Pepsin - 48H @ 37°C (as well as Pepsin - 48H @ 37°C (2))	Pepsin - 48H @ 37°C (as well as Pepsin - 48H @ 37°C (2))
Pepsin (as well as Pepsin (2))	Pepsin (as well as Pepsin (2))
Poly(vinyl) butyral resin [in 1:1 acetone:IMS]	
Potassium carbonate	
Potassium carbonate - 6H @ 80°C	Potassium carbonate - 6H @ 80°C
PVAC	
PVAC/PVAL	PVAC/PVAL
Shellac [in ethanol]	
Sodium bicarbonate - 24H @ 80°C (as well as Sodium bicarbonate - 24H @ 80°C (2))	Sodium bicarbonate - 24H @ 80°C (as well as Sodium bicarbonate - 24H @ 80°C (2))
Sodium bicarbonate (as well as Sodium bicarbonate (2) and re-ppt)	Sodium bicarbonate (2)
Sodium carbonate	
Sodium chloride - 24H @ 37° C	
Sodium hydroxide	Sodium hydroxide
Sodium hypochlorite (as well as Sodium hypochlorite (2) and re-ppt presumed damaging)	Sodium hypochlorite (as well as Sodium hypochlorite (2) and re-ppt presumed damaging)
Sodium perborate column cleaned as well as re-ppt (Sodium perborate and Sodium perborate (2), Sodium perborate - O/N from 100°C to RT and Sodium perborate - O/N from 100°C to RT (2) presumed damaging)	Sodium perborate column cleaned as well as re-ppt (Sodium perborate and Sodium perborate (2), Sodium perborate - O/N from 100°C to RT and Sodium perborate - O/N from 100°C to RT (2) presumed damaging)
Sodium sulfide [in saline]	Sodium sulfide [in saline]
Sodium sulfide - 6H @ 80°C [in saline]	Sodium sulfide - 6H @ 80°C [in saline]
Turpentine	Turpentine
Water (control) - 24H @ 80°C (2)	Water (control) - 24H @ 80°C (2)
Water (control) - 40H @ 80°C (as well as Water (control) - 40H @ 80°C (2))	Water (control) - 40H @ 80°C (as well as Water (control) - 40H @ 80°C (2))
Water (control) - 48H @ 37°C	Water (control) - 48H @ 37°C
Water (control) - 6H @ 80°C	Water (control) - 6H @ 80°C
Water (control) - column cleaned	
Water (control) - O/N from 100°C to RT (as well as Water (control) - O/N from 100°C to RT (2))	Water (control) - O/N from 100°C to RT (as well as Water (control) - O/N from 100°C to RT (2))
White spirit	White spirit

Table 15. Summary of treatments unsafe to use based on this study. "H" = hours, "O/N" = overnight, "RT" = room temperature.

Although some tentative predictions of the potential effects of treatments could be made based on previous studies, such as discussions of DNA damage (Lindahl 1993), Brown's (1999) list of safe and unsafe chemicals as well as some experimental published results (Kigawa et al. 2003; Williams 1999), due to inconsistencies among these sources, the effects of the majority of treatments were unanticipated at the start of this research.

Initial visual inspection of the scatterplots and boxplots produced using the peak height ratio data from the treatments tested in the screening procedure suggest that although the bulk of the treatment ratios were clustered around the water control, a few treatments were particularly damaging to DNA. Sodium sulfide, turpentine, and treatments heated to 80°C or higher, resulted in almost complete destruction of both the TAT and SRY 4064 DNA fragments. Pepsin, acetic acid and mineral oil were only slightly less damaging, but their ratios were still well below that of the water control and the majority of other treatments. That the most damaging treatments would include acetic acid and heat was not surprising, as it is known that DNA is susceptible to damage by both acids and heat (Lindahl 1993). Pepsin was not expected

to damage DNA, as it is routinely used in some digestion protocols (Hedley et al. 1983; Mørkve and Laerum 1991), however, as pepsin originates from hog stomach, impurities including deoxyribonucleases were possibly present and caused the damage observed. Turpentine was predicted by Brown (1999) to be safe to use, but was one of the most damaging *in vitro* treatments tested in this study. This may be because the turpentine used was a proprietary product, and not of high quality, which may have contained any number of impurities, however it is quite possible that such materials would have been used in the past. Initially, mineral oil was not expected to be damaging, as it is routinely used in some DNA research laboratories to minimise evaporation in PCR samples, but preliminary studies found it to be quite damaging to DNA, so it was included in the study as a "damage control". It was thought that the mineral oil result was due to either contamination or the presence of oxidation products in the mineral oil stock used, however, further investigation into the effects of mineral oil using samples from other laboratories were inconclusive (data not presented), so a separate study into the matter is suggested, as is reconsideration of the use of mineral oil in DNA samples during long-term storage.

Hydrogen peroxide, carbon tetrachloride, mercury (II) chloride (mercury salts) and sodium hydroxide were considered unsafe by Brown (1999), and were found to be damaging in this study, as well. Brown (1999) also suggested that ammonium hydroxide was unsafe, but it was only found to be unsafe for the TAT fragment.

In addition to turpentine, gasoline, white spirit (naphtha) and benzene were considered by Brown (1999) to be safe for DNA. However, this study found that they were all damaging. Linseed oil in turpentine (mixed in equal parts) was also found to be damaging, but considerably less damaging than turpentine alone.

Heated treatments were expected to be more damaging than their unheated replicates, as heat is known to be damaging to DNA (Lindahl 1993). In the heated water control samples, heating to 100°C for 1 minute or heating to 80°C for 6 hours was found to be damaging, and after heating to 80°C for 24 hours, DNA was effectively destroyed. Treatments heated to 80°C consistently suffered high levels of damage, and peak height ratio damage was missing, making statistical analysis impossible. However, the lack of statistical evidence is not deemed important, as the extreme nature of the damage sustained was conspicuous. Although the TAT fragment in unheated detergent samples was damaged, both fragments in detergent samples heated to 80°C for 40 hours were consistently destroyed. Similarly, in most cases sodium bicarbonate samples heated to 80°C for 24 hours were also more damaged than the unheated sodium bicarbonate samples, and pepsin samples heated to 37°C for 48 hours were more damaged than the unheated pepsin samples. Potassium carbonate samples heated to 80°C for 6 hours were significantly

more damaged than unheated potassium carbonate samples. Heating sodium chloride to 37°C for 24 hours was damaging to the TAT fragment, only, but was comparable to the unheated sodium chloride samples and water control for the SRY 4064 fragment. Insufficient data were obtained to quantitatively or semi-quantitatively assess the effect of heating sodium perborate to 100°C and allowing it to cool to room temperature, but based upon the damaging effect heating to 100°C had on the water control, it is assumed this treatment would be damaging, as well. The only heated treatment that damaged DNA less than its unheated replicate was sodium sulfide heated to 80°C for 6 hours. This is possibly due to the decomposition of sodium sulfide at this temperature and a subsequent decrease in reactivity.

In order to assess the effects of chemicals dissolved in organic solvents, comparison of the mixed treatment against the solvent alone, in addition to comparison against the water control, was necessary. Shellac in acetone appeared slightly more damaging to the TAT fragment than acetone alone (64.2 ±17.8% and 95.3 ±8.9%, total TY average and standard deviation of DNA preservation respectively) however, insufficient data were gathered to calculate any statistical significance.

Cellulose nitrate in 1:1 ether:ethanol was found to be damaging to the TAT fragment only. This treatment appeared only slightly more damaging than 1:1 ether:ethanol alone in the scatterplots and boxplots. Only a small difference was found between the two treatments in the percent DNA preservation calculations: cellulose nitrate in 1:1 ether:ethanol = 84.0 ± 11.6% and 1:1 ether:ethanol = 92.2 ± 11.2% total TY average and standard deviation. This difference was statistically significant for only one of the ten TY ratios, TYE2: U = 1, p = .043 (asymptotic significance) (cellulose nitrate in 1:1 ether:ethanol n = 4 and median = 3.484, 1:1 ether:ethanol n = 4 and median = 4.261).

Linseed oil diluted by 50% with turpentine (overall preservation = 76.7 ±18.9%) was considerably less damaging than turpentine alone (overall preservation = 8.6 ± 2.8%). Although no statistics could be calculated for the SY peak height ratios, the difference observed was statistically significant for all ten TY peak height ratios. For all TY ratios, U = 0 and p = .029 (exact significance) and the medians varied by up to 95% (linseed oil in turpentine n = 4 and median = 1.467, 1.512, 2.222, 2.196, 1.768, 1.815, 1.709, 1.694, 4.429 and 4.412; turpentine n = 4 and median = 0.217, 0.220, 0.321, 0.319, 0.149, 0.131, 0.138, 0.125, 0.241 and 0.209 (TYA1-TYE2)).

Gasoline (mixed 93:16:1 gasoline:ethanol:turpentine) was damaging to both the TAT and SRY 4064 fragments. Turpentine (overall preservation = 8.6 ± 2.8%) was considerably more damaging than gasoline (overall preservation = 75.3 ± 8.7%), which was more damaging than

ethanol (overall preservation = 91.7 ± 11.9%). Each of these differences was found to be statistically significant for the majority of ratios with sufficient data for statistical calculations to be made (no statistics could be calculated for the SM ratio comparison of turpentine and gasoline). When comparing peak height ratio data from the turpentine and gasoline treated samples, it became clear that gasoline was considerably less damaging than turpentine, as the Mann-Whitney U = 0 and p = .029 (exact significance) for all ten TY ratios, the medians of which varied by up to 96% (gasoline n = 4 and median = 1.414, 1.440, 2.028, 2.060, 1.712, 1.732, 1.642, 1.653, 3.373 and 3.503 (TYA1-TYE2); turpentine n = 4 and median = 0.217, 0.220, 0.321, 0.319, 0.149, 0.131, 0.138, 0.125, 0.241 and 0.209 (TYA1-TYE2)). Comparisons of peak height ratio data from the gasoline and ethanol treated samples were similarly significant, with U = 0 and p = .029 (exact significance) for all 4 SM ratios and 6 TY ratios, but the medians only varied up to approximately 32% (ethanol n = 4 and median = 0.956, 0.963, 0.441 and 0.419 (SMA1-SMB2), 1.608, 1.622, 2.343, 2.339, 1.945 and 2.059 (TYA1-TYC2); gasoline n = 4 and median = 0.672, 0.656, 0.312 and 0.294 (SMA1-SMB2), 1.414, 1.440, 2.028, 2.060, 1.712 and 1.732 (TYA1-TYC2). Although the addition of turpentine to gasoline may have increased the damaging effect of this treatment, it is unlikely that 1% turpentine can account for all the damage induced, as a 50% solution (linseed oil in turpentine) was slightly less damaging.

Methylmethacrylate/ethacrylate in acetone was more damaging than acetone alone for the TAT fragment (86.7 ± 7.3% and 95.3 ± 8.9% overall TY preservation, respectively). This difference was found to be significantly damaging for two TY ratios. For the TYE1 ratio, U = 0, p = .021 (exact significance) (methylmethacrylate/ethacrylate in acetone n = 4, median = 3.358; acetone n = 4, median = 3.937) and for the TYE2 ratio, U = 1, p = .043 (asymptotic significance) (methylmethacrylate/ethacrylate in acetone n = 4, median = 3.298; acetone n = 4, median = 3.999).

Poly(vinyl) butyral resin in 1:1 acetone:IMS was observed to be somewhat more damaging than 1:1 acetone:IMS for the TAT fragment (69.9 ± 8.0% and 91.8 ±8.3% overall TY preservation, respectively). This difference was found to be statistically significant for 5 TY ratios. For TYA1, TYA2 and TYC2, U = 0, p = .029 (exact significance) ((poly(vinyl) butyral resin in 1:1 acetone:IMS n = 4, median = 1.383, 1.486 and 1.710; 1:1 acetone:IMS n = 4, median = 1.713, 1.725 and 2.168), and TYE1 and TYE2, U = 1, p =.043 (asymptotic significance) (poly(vinyl) butyral resin in 1:1 acetone:IMS n = 4, median = 2.912 and 3.267; 1:1 acetone:IMS n = 4, median = 3.678 and 3.778).

Although the PVAC and PVAC/PVAL compounds tested were from different sources, and therefore cannot necessarily be directly compared in the same way other mixed treatments were

compared in this study, there was a conspicuous difference observed in the boxplots and scatterplots, with PVAC/PVAL consistently appearing more damaging than PVAC alone. The overall preservation of PVAC was 88.1 ± 14.6% and the overall preservation of PVAC/PVAL was 75.7 ± 14.0%. This difference was statistically significant for all SM ratios and for 5 TY ratios. For all ratios, and for both PVAC and PVAC/PVAL, n = 4. Statistical data generated for the SM peak height ratios were as follows: SMA1 U = 0, p = .029 (exact significance) (PVAC median = 0.985, PVAC/PVAL median = 0.842); SMA2 U = 1, p = .043 (asymptotic significance) (PVAC median = 0.934, PVAC/PVAL median = 0.850); SMB1 U = 0, p = .029 (exact significance) (PVAC median = 0.445, PVAC/PVAL median = 0.387); SMB2 U = .5, p = .029 (asymptotic significance) (PVAC median = 0.416, PVAC/PVAL median = 0.381). Statistical data generated for the TY peak height ratios were as follows: TYA1 U = 0, p = .029 (exact significance) (PVAC median = 1.532, PVAC/PVAL median = 1.204); TYB1 U = 0, p = .029 (exact significance) (PVAC median = 2.206, PVAC/PVAL median = 1.705); TYB2 U = 1, p = .043 (asymptotic significance) (PVAC median = 2.265, PVAC/PVAL median = 1.861); TYC1 U = 0, p = .029 (exact significance) (PVAC median = 1.780, PVAC/PVAL median = 1.422); and TYD1 U = 0, p = .029 (exact significance) (PVAC median = 1.734, PVAC/PVAL median = 1.400). It should also be noted here that both PVAC and PVAC/PVAL compounds tested were proprietary products, the exact chemical composition of which was not available. Therefore, any number of impurities may have been present, and a purer conservation grade compound may have yielded a different result. The use of proprietary PVAC and PVAC/PVAL compounds has been widespread amongst field archaeologists in the past, contrary to advice given by conservators. This result further reinforces the suggestion that their use should be avoided.

Although a conservative, conservation based view as to what constitutes an unsafe treatment is used here, whether or not these treatments are damaging enough to prevent DNA extraction and amplification, or if their use would inhibit PCR is unknown. As the interaction of chemical treatments with DNA *in vitro* cannot replicate the conditions experienced by an object or specimen under conservation treatment, testing whether treatments damage DNA beyond the sensitivity of PCR falls outside the scope of this study. However, this could be tested in the future (see section 6.5).

5.1.8. Treatments found to be safe for DNA in the screening test

Twenty-seven treatments tested were considered safe for the 225bp SRY 4064 fragment, whereas only 13 treatments tested were considered safe for the 112bp TAT fragment (see Table 16 for a full summary) based upon the boxplot/scatterplot scoring system and Mann-Whitney test results. Upon comparison of the safe lists for each fragment against the percentage of DNA preserved, it was observed that those treatments deemed safe all demonstrated greater than 90% DNA preservation. This is considered the lowest acceptable degree of DNA preservation to be

designated as a safe treatment, although other factors may also require consideration, as discussed below.

Repeated treatment samples or additional clean-up samples also considered safe were listed with the original treatment rather than being counted as a separate treatment. Also not included in the safe treatment list was the column cleaned water control, which was not damaging for the SRY 4064 fragment, as well as two other water control tests, namely those which were ether extracted and unmixed/unheated, both of which were not damaging to either fragment. In cases that the boxplot/scatterplot scoring system deemed a treatment safe, but the Mann-Whitney statistic deemed the treatment unsafe, the treatment was ultimately considered unsafe. This applied to cellulose nitrate, methylmethacrylate/ethacrylate and heating sodium chloride for 48 hours to 37°C for the TAT fragment and to heating water for 48 hours to 37°C (2) for the SRY 4064 fragment. Typically, in cases that insufficient data was collected to calculate a Mann-Whitney statistic for a treatment, the boxplot/scatterplot scoring system deemed such treatments unsafe. A few exceptions were found, all with respect to their effect on the SRY 4064 fragment. These treatments included: detergent (2), detergent (re-ppt), EDTA (2), EDTA (re-ppt), and gum arabic. The safety of these treatments was also questioned when the percentage DNA preserved data was analysed.

The percentage DNA preserved data was used to further investigate the safe treatment lists based on the boxplot/scatterplot scoring system and Mann-Whitney statistics for both DNA fragments tested. All of the treatments deemed safe for the TAT fragment had an average percentage of DNA preservation greater than 90%, and the lower limit of the standard deviation was never less than 80%. As mentioned previously, when calculating the average damage ratio for the control samples, the average standard deviation was roughly 10-12%. Of those treatments on the TAT safe list, two treatments exceeded a standard deviation of this value, namely water heated for 48 hours to 37°C (2), with a standard deviation of 36.8%, and arsenic trioxide, with a standard deviation of 13.4%. It was decided that those treatments with average DNA preservation greater than 90% and standard deviations exceeding 12%, but maintaining a lower limit of DNA preservation greater than 70% would be included on the initial lists of potentially safe treatments, but given questionable status (such treatments are italicised in Table 16), as it was often these treatments that lacked some sample data and future analyses may better assess their effects. Several treatments on the SRY 4064 potentially safe list were found to have questionable status, as well, and such treatments were always considered unsafe for the TAT fragment.

Treatments potentially safe based on TY results	Treatments potentially safe based on SM results
1:1 acetone:IMS	1:1 acetone:IMS
1:1 ethanol:ether	1:1 ethanol:ether

Treatments potentially safe based on TY results	Treatments potentially safe based on SM results
Acetone	Acetone
	Acrylic emulsion
	Ammonium hydroxide
Amyl acetate	Amyl acetate
Arsenic trioxide	Arsenic trioxide
	Cellulose nitrate [in 1:1 ethanol:ether]
Chloroform	Chloroform
	Detergent (as well as Detergent (2), and re-ppt)
	EDTA (as well as EDTA (2), and re-ppt)
	Ethanol
Ethyl acetate	Ethyl acetate
	Gum arabic (as well as re-ppt)
IMS	IMS
	Methylmethacrylate/ethylacrylate [in acetone]
	Poly(vinyl) butyral resin [in 1:1 acetone:IMS]
	Potassium carbonate
	PVAC
	Shellac [in ethanol]
	Sodium bicarbonate (as well as re-ppt)
	Sodium carbonate
Sodium chloride	Sodium chloride
	Sodium chloride - 24H @ 37°C
Toluene	Toluene
Trichloroethylene	Trichloroethylene
Water (control) - 48H @ 37°C (2)	
Xylene	Xylene

Table 16. Summary of treatments potentially safe to use based on the results of this study. Treatments in italics are considered questionably safe, and further verification of their effects is needed. "H" = hours.

A final safe list of treatments was compiled based on the most conservative interpretation of the experimental results of this study. Using the overall percent DNA preserved calculations (the average of all ratios) for treatments with data for both the TAT and SRY 4064 fragments, and the criteria of an average DNA preservation of greater than 90% with a standard deviation less than 12% and a lower limit of DNA preservation of 80%, the list is, effectively, those treatments safe for use on the TAT fragment that were also safe for the SRY 4064 fragment. With the exception of heating water for 48 hours to 37°C, all other treatments deemed safe for the TAT fragment are also safe for the SRY 4064 fragment (the final, conservative safe list is presented in Table 17). Heating water for 48 hours to 37°C is not included in the list of treatments safe for the SRY 4064 fragment, because although it appeared safe in the boxplots and scatterplots, it was deemed unsafe using the Mann-Whitney test, due to the SMB2 ratio having a U value = 0, $p = .029$ (exact significance calculated), median of water (control) (2) = 0.454 (n = 4), median of water heated to 37°C for 48 hours (2) = 0.335 (n = 4). This may be due to the small sample size, or the effect of mixing on the normal water control (see below).

Safe treatments	Overall % average DNA preservation	Overall % standard deviation
IMS	100.4	8.4
Trichloroethylene	100.4	8.9
Sodium chloride	100.4	11.7
Toluene	99.1	8.1
Amyl acetate	97.7	8.7

Safe treatments	Overall % average DNA preservation	Overall % standard deviation
Acetone	97.5	8.4
Chloroform	96.6	9
Xylene	96.3	10
Arsenic trioxide	96.2	11.5
1:1 acetone:IMS	96	9.9
1:1 ethanol:ether	95.5	10.9
Ethyl acetate	94.4	10.2

Table 17. Final safe list of treatments based on the results of this study.

Most of the treatments deemed safe for use on the TAT fragment were organic solvents. With the exception of heating water for 48 hours to 37°C, sodium chloride (physiological saline solution) and arsenic trioxide, all other treatments considered potentially safe for use on the TAT fragment were organic solvent-based, suggesting that the TAT fragment is more susceptible to hydrolytic damage than the SRY 4064 fragment. Of the 27 treatments deemed safe for use on the SRY 4064 fragment, only 12 are water soluble, indicating a greater resistance to hydrolytic damage. It should be noted that both chloroform and xylene evaporated overnight, therefore these treatments can only be deemed safe for short treatment periods, and their effects if used for the full exposure time remain unknown. However, the use of organic solvents alone on hard and soft tissues is uncommon (with the exception of degreasing); organic solvents typically serve as a liquid medium for another material, such as a consolidant or pesticide. Additionally, organic solvents are usually a second choice for many conservators on health and safety grounds, thereby making aqueous treatments much more common. Based on these results, it may be concluded that most aqueous conservation treatments, and potentially even washing, will result in damage. This means that including sampling prior to chemical intervention into conservation treatment strategies is of great importance.

Compared to the normal water control the unmixed/unheated water control was positively preserved (overall preservation = 111.7 ± 11.7%), with median values higher than the normal water control for all but one ratio (TYD2), and the difference being statistically significant for one SM ratio and five of the ten TY ratios. This makes the unmixed/unheated water control the only treatment that was statistically significantly positively preserving for both the TAT and SRY 4064 fragments and with the highest number of positively preserving significant statistics for any treatment, which was in line with the boxplot and scatterplot observations. For all statistics calculated, n = 4 for the normal water control and n = 3 for the unmixed/unheated water control. The significant statistics calculated for the SMB2 were U = 0.5 and p = .050 (asymptotic significance), normal water control median = 0.454 and the unmixed/unheated water control median = 0.508. The significant statistics calculated for the TY ratios were as follows: TYA1 U = 0 and p = .034 (asymptotic significance), normal water control median = 1.545 and unmixed/unheated water control median = 1.720; TYA2 U = 0 and p = .034 (asymptotic significance), normal water control median = 1.594 and unmixed/unheated water

control median = 1.825; TYB2 U = 0 and p = .034 (asymptotic significance), normal water control median = 2.277 and unmixed/unheated water control median = 2.625; TYC1 U = 0 and p = .034 (asymptotic significance), normal water control median = 1.056 and unmixed/unheated water control median = 1.223; TYC2 U = 0 and p = .034 (asymptotic significance), normal water control median = 1.077 and unmixed/unheated water control median = 1.213. This implies that the TAT fragments are particularly susceptible to mechanical damage resulting from being on a mixer at a relatively low setting for 7 days. Although this result does not have an effect on the future of conservation treatments, as a specimen is unlikely to be placed on a mixer for conservation treatment and DNA in a specimen would not be free to move about, it is potentially important to note when devising biochemical laboratory procedures. Any damage in this study resulting from mixing was equalised across treatment samples, as all (unheated) samples were placed on a mixer for the duration of the treatment exposure time, and all heated samples (both treatments and water controls) were unmixed, so this observation only affects comparisons of heated water control samples against the normal water control (which may account for some of the preservative effect noted for the water control heated for 48 hours to 37°C for the TAT samples), but otherwise does not change any of the results reported here. Several other treatments also appeared to positively preserve either the TAT or SRY 4064 fragment, but not both.

In addition to the unmixed/unheated water control, only 4 other treatments appeared to enhance the preservation of the TAT fragments. The data for each of these treatments showed a trend of the treatment medians being higher than that of the water control for at least 50% of the TY ratios. Those treatments which appeared to preserve DNA were: amyl acetate (median higher than water control for 9 out of 10 ratios), IMS (7 out of 10), xylene (5 out of 10) and the water control heated for 48 hours to 37°C (6 out of 10; repeated samples only).

Similar to the unmixed/unheated water control, 8 of the 9 treatments that appeared to enhance the preservation of the SRY 4064 fragments consistently had higher medians than the water control for all of the SM ratios calculated. These treatments included: poly(vinyl) butyral resin, potassium carbonate, PVAC, shellac, sodium bicarbonate, sodium carbonate and both heated and unheated sodium chloride (all of these treatments had 4 SM ratio statistics calculated, with the exception of sodium bicarbonate, for which only 2 ratios had sufficient data for statistical analysis). Of these treatments, potassium carbonate had the most preserving effect (205.0 ± 29.4% SM preservation), with all four ratios being statistically significant at p = .001 or less (exact significance). Sodium carbonate was only slightly less preserving (145.1 ± 14.9% SM preservation), with all four ratios statistically significant at p = .007 (exact significance) or less. Only one out of three ratio medians for EDTA was higher than the water control (121.2 ± 49.5%), and as this difference was quite large (median of water control = 0.384, median for

EDTA = 0.600), it was calculated as statistically significant. However, due to the small sample size (EDTA n = 2), and the lack of consistency within the other SM ratios, this result is considered somewhat unreliable.

Statistically significant Mann-Whitney data for all treatments with a preserving effect are presented in Table 18. All statistics calculated are included in Appendix B.

Peak	Treatment	N	Median	Test statistics	
TYD1	Water (control)	14	1.809	Mann-Whitney U	7
	Amyl acetate	4	2.068	Asymp. Sig. (2-tailed)	.026
	Total	18		Exact Sig. (2-tailed)	.025
SMB2	Water (control)	14	0.384	Mann-Whitney U	0
	EDTA	2	0.600	Asymp. Sig. (2-tailed)	.026
	Total	16		Exact Sig. (2-tailed)	.017
TYD1	Water (control)	14	1.809	Mann-Whitney U	8
	IMS	4	2.088	Asymp. Sig. (2-tailed)	.034
	Total	18		Exact Sig. (2-tailed)	.035
TYD2	Water (control)	14	1.832	Mann-Whitney U	8
	IMS	4	2.146	Asymp. Sig. (2-tailed)	.034
	Total	18		Exact Sig. (2-tailed)	.035
SMB1	Water (control)	14	0.393	Mann-Whitney U	9
	Poly(vinyl) butyral resin	4	0.479	Asymp. Sig. (2-tailed)	.044
	Total	18		Exact Sig. (2-tailed)	.046
SMA1	Water (control)	14	0.934	Mann-Whitney U	0
	Potassium carbonate	4	1.596	Asymp. Sig. (2-tailed)	.003
	Total	18		Exact Sig. (2-tailed)	.000
SMA2	Water (control)	14	0.897	Mann-Whitney U	0
	Potassium carbonate	4	1.660	Asymp. Sig. (2-tailed)	.003
	Total	18		Exact Sig. (2-tailed)	.001
SMB1	Water (control)	14	0.393	Mann-Whitney U	0
	Potassium carbonate	4	0.912	Asymp. Sig. (2-tailed)	.003
	Total	18		Exact Sig. (2-tailed)	.001
SMB2	Water (control)	14	0.384	Mann-Whitney U	0
	Potassium carbonate	4	0.921	Asymp. Sig. (2-tailed)	.003
	Total	18		Exact Sig. (2-tailed)	.001
SMB1	Water (control)	14	0.393	Mann-Whitney U	8
	PVAC	4	0.445	Asymp. Sig. (2-tailed)	.034
	Total	18		Exact Sig. (2-tailed)	.035
SMB1	Water (control)	14	0.393	Mann-Whitney U	0
	Shellac	2	0.637	Asymp. Sig. (2-tailed)	.026
	Total	16		Exact Sig. (2-tailed)	.017
SMB1	Water (control)	14	0.393	Mann-Whitney U	0
	Sodium bicarbonate	2	1.062	Asymp. Sig. (2-tailed)	.026
	Total	16		Exact Sig. (2-tailed)	.017
SMA1	Water (control)	14	0.934	Mann-Whitney U	4
	Sodium carbonate	4	1.138	Asymp. Sig. (2-tailed)	.011
	Total	18		Exact Sig. (2-tailed)	.007
SMA2	Water (control)	14	0.897	Mann-Whitney U	2
	Sodium carbonate	4	1.129	Asymp. Sig. (2-tailed)	.006
	Total	18		Exact Sig. (2-tailed)	.003
SMB1	Water (control)	14	0.393	Mann-Whitney U	1
	Sodium carbonate	4	0.609	Asymp. Sig. (2-tailed)	.004
	Total	18		Exact Sig. (2-tailed)	.001
SMB2	Water (control)	14	0.384	Mann-Whitney U	3
	Sodium carbonate	4	0.641	Asymp. Sig. (2-tailed)	.008
	Total	18		Exact Sig. (2-tailed)	.005
SMA2	Water (control)	14	0.897	Mann-Whitney U	5
	Sodium chloride	4	1.030	Asymp. Sig. (2-tailed)	.015
	Total	18		Exact Sig. (2-tailed)	.012

Peak	Treatment	N	Median	Test statistics	
SMB2	Water (control)	14	0.384	Mann-Whitney U	8
	Sodium chloride	4	0.455	Asymp. Sig. (2-tailed)	.034
	Total	18		Exact Sig. (2-tailed)	.035
SMB2	Water (control)	14	0.384	Mann-Whitney U	2
	Sodium chloride - 24H @ 37°C	5	0.538	Asymp. Sig. (2-tailed)	.002
	Total	19		Exact Sig. (2-tailed)	.001
TYD1	Water (control)	14	1.809	Mann-Whitney U	9
	Xylene	4	2.056	Asymp. Sig. (2-tailed)	.044
	Total	18		Exact Sig. (2-tailed)	.046
TYC2	Water (control) (2)	4	1.077	Mann-Whitney U	0
	Water (control) - 48H @ 37°C (2)	4	1.853	Asymp. Sig. (2-tailed)	.021
	Total	8		Exact Sig. (2-tailed)	.029
TYE2	Water (control) (2)	4	1.642	Mann-Whitney U	0
	Water (control) - 48H @ 37°C (2)	4	3.454	Asymp. Sig. (2-tailed)	.021
	Total	8		Exact Sig. (2-tailed)	.029
SMB2	Water (control) (2)	4	0.454	Mann-Whitney U	.5
	Water (control) - unheated, unmixed	3	0.508	Asymp. Sig. (2-tailed)	.050
	Total	7		Exact Sig. (2-tailed)	.086
TYA1	Water (control) (2)	4	1.545	Mann-Whitney U	0
	Water (control) - unheated, unmixed	3	1.720	Asymp. Sig. (2-tailed)	.034
	Total	7		Exact Sig. (2-tailed)	.057
TYA2	Water (control) (2)	4	1.594	Mann-Whitney U	0
	Water (control) - unheated, unmixed	3	1.825	Asymp. Sig. (2-tailed)	.034
	Total	7		Exact Sig. (2-tailed)	.057
TYB2	Water (control) (2)	4	2.277	Mann-Whitney U	0
	Water (control) - unheated, unmixed	3	2.625	Asymp. Sig. (2-tailed)	.034
	Total	7		Exact Sig. (2-tailed)	.057
TYC1	Water (control) (2)	4	1.056	Mann-Whitney U	0
	Water (control) - unheated, unmixed	3	1.223	Asymp. Sig. (2-tailed)	.034
	Total	7		Exact Sig. (2-tailed)	.057
TYC2	Water (control) (2)	4	1.077	Mann-Whitney U	0
	Water (control) - unheated, unmixed	3	1.213	Asymp. Sig. (2-tailed)	.034
	Total	7		Exact Sig. (2-tailed)	.057

Table 18. Mann-Whitney statistical data for treatments that appear to preserve DNA, $.05 \leq p < .01$, $.01 \leq p < .001$, and $.001 \leq p < .000$. Extracted from Appendix B.

5.1.9. Wilcoxon signed ranks test results

The Wilcoxon signed ranks test was used to assess differences between peak height ratios on repeated electrophoresis runs of sample data. The Wilcoxon signed ranks test was chosen because it is suitable for non-parametric data, and is routinely used to compare repeated data from the same participant or sample. Two-tailed significance was used, as there was no expectation prior to running the test as to whether a positive or negative change should be seen in the data (all things being equal, no change was expected). Wilcoxon statistics were calculated for each peak height. Exact significance is referred to if calculated, but due to the large sample size for this test, asymptotic significance could only be calculated for some datasets.

The Wilcoxon signed ranks test was performed initially on a dataset containing all repeated samples which had been frozen prior to sample processing and first electrophoresis run. A subset of treatments were repeated and not frozen prior to electrophoresis. Sample data was further split into groups for analysis based on treatment type (i.e. aqueous or organic solvent-based).

When the data from the samples that were frozen prior to sample preparation and electrophoresis were used, differences between peak height ratio data were calculated to be statistically significant for six out of seven peak height ratios. However, due to the large sample size, although there was a consistent variation between sample runs leading to a statistical significance, the difference between means was typically quite small. For the SRY 4064 fragment, the peak height ratio of SMA2 (median = 0.840) was statistically significantly lower than SMA1 (median = 0.844), $T = 4462$, $p = .000$ (exact significance) and the peak ratio of SMB2 (median = 0.386) was significantly lower than SMB1 (median = 0.394), $T = 4635$, $p = .001$ (exact significance). Although this suggests that the SRY 4064 fragments were more damaged in the second run, than in the first run, the difference in median is slight, 0.004 for the SMA peak height ratio and 0.008 for the SMB peak height ratio. Of the TAT fragment peak height ratios, only the TYC ratio displayed a similar pattern, with TYC2 (median = 1.611) statistically significantly lower than TYC1 (median = 1.635), $T = 5825$, $p = .004$ (asymptotic significance). Conversely, the peak ratio of TYA2 (median = 1.524) was statistically significantly higher than TYA1 (median = 1.486), $T = 5485$, $p = .000$ (asymptotic significance); TYB2 (median = 2.181) was significantly higher than TYB1 (2.157), $T = 5207$, $p = .000$ (asymptotic significance); and TYE1 (median = 3.071) was significantly higher than TYE2 (median = 3.097), $T = 6101$, $p = .017$. Only the TYD peak ratio exhibited no significant change between runs, $T = 6720$, $p = .273$ (exact significance). See Table 19 for a full summary of the statistical test results.

Wilcoxon signed ranks		N	Mean Rank	Sum of Ranks	Medians		Test statistics	
SMA2 - SMA1	Negative Ranks	108(a)	90.13	9,734.00	SMA1 median	0.844	Z(a)	-4.175
	Positive Ranks	60(b)	74.37	4,462.00	SMA2 median	0.840	Asymp. Sig. (2-tailed)	.000
	Ties	0(c)					Exact Sig. (2-tailed)	.000
	Total	168						
SMB2 - SMB1	Negative Ranks	104(d)	83.95	8,731.00	SMB1 median	0.394	Z(a)	-3.393
	Positive Ranks	59(e)	78.56	4,635.00	SMB2 median	0.386	Asymp. Sig. (2-tailed)	.001
	Ties	0(f)					Exact Sig. (2-tailed)	.001
	Total	163						
TYA2 - TYA1	Negative Ranks	70(g)	78.36	5,485.00	TYA1 median	1.486	Z(b)	-3.702
	Positive Ranks	109(h)	97.48	10,625.00	TYA2 median	1.524	Asymp. Sig. (2-tailed)	.000
	Ties	0(i)					Exact Sig. (2-tailed)	(c)
	Total	179						
TYB2 - TYB1	Negative Ranks	69(j)	75.46	5,207.00	TYB1 median	2.157	Z(b)	-3.514
	Positive Ranks	104(k)	94.65	9,844.00	TYB2 median	2.181	Asymp. Sig. (2-tailed)	.000
	Ties	1(l)					Exact Sig. (2-tailed)	(c)
	Total	174						
TYC2 - TYC1	Negative Ranks	67(m)	86.94	5,825.00	TYC1 median	1.635	Z(b)	-2.900
	Positive Ranks	109(n)	89.46	9,751.00	TYC2 median	1.611	Asymp. Sig. (2-tailed)	.004
	Ties	0(o)					Exact Sig. (2-tailed)	(c)
	Total	176						
TYD2 - TYD1	Negative Ranks	79(p)	85.06	6,720.00	TYD1 median	1.502	Z(b)	-1.099
	Positive Ranks	93(q)	87.72	8,158.00	TYD2 median	1.467	Asymp. Sig. (2-tailed)	.272
	Ties	0(r)					Exact Sig. (2-tailed)	.273
	Total	172						
TYE2 - TYE1	Negative Ranks	70(s)	87.16	6,101.00	TYE1 median	3.071	Z(b)	-2.382

Wilcoxon signed ranks		N	Mean Rank	Sum of Ranks	Medians		Test statistics	
	Positive Ranks	105(t)	88.56	9,299.00	TYE2 median	3.097	Asymp. Sig. (2-tailed)	.017
	Ties	0(u)					Exact Sig. (2-tailed)	(c)
	Total	175						
a. SMA2 < SMA1; b. SMA2 > SMA1; c. SMA2 = SMA1; d. SMB2 < SMB1; e. SMB2 > SMB1; f. SMB2 = SMB1; g. TYA2 < TYA1; h. TYA2 > TYA1; i. TYA2 = TYA1; j. TYB2 < TYB1; k. TYB2 > TYB1; l. TYB2 = TYB1; m. TYC2 < TYC1; n. TYC2 > TYC1; o. TYC2 = TYC1; p. TYD2 < TYD1; q. TYD2 > TYD1; r. TYD2 = TYD1; s. TYE2 < TYE1; t. TYE2 > TYE1; u. TYE2 = TYE1							a. Based on positive ranks; b. Based on negative ranks; c. Some or all exact significances cannot be computed because there is insufficient memory.	

Table 19. Summary of Wilcoxon signed ranks test for samples that were frozen prior to sample preparation and first electrophoresis run, $.05 \leq p < .01$, $.01 \leq p < .001$, and $.001 \leq p < .000$.

When the Wilcoxon signed ranks test was run on the same samples split into smaller groups based on treatment type (water soluble treatments or organic solvent-based treatments), it became clear that the effect described above was predominantly due to the organic solvent-based treatments. Only one peak in the water soluble treatment group demonstrated a statistically significant change between runs (TYE2 (median = 1.690) was significantly greater than TYE1 (median = 1.562), $T = 2503$, $p = .040$) (See Table 20). However, the organic solvent-based treatments largely mimic the trends mentioned above when samples were not split into treatment types (see Table 21). SMA2 (median = 0.844) was statistically significantly lower than SMA1 (median = 0.881), $T = 755$, $p = .000$ (exact significance) and SMB2 (median = 0.381) was significantly lower than SMB1 (median = 0.395), $T = 892$, $p = .000$ (exact significance). Conversely, TYA2 (median = 1.629) was significantly higher than TYA1 (median = 1.612), $T = 1520$, $p = .003$ (exact significance); TYB2 (median = 2.390) was significantly higher than TYB1 (median = 2.315), $T = 1343$, $p = .003$ (exact significance); TYC2 (median = 2.040) was significantly higher than TYC1 (median = 1.949), $T = 1248$, $p = .000$ (exact significance); TYE2 (median = 3.835) was significantly higher than TYE1 (median = 3.687), $T = 1480$, $p = .002$ (exact significance). Only the TYD peak ratio exhibited no significant change between runs, $T = 1797$, $p = .052$ (TYD2 median = 1.827, TYD1 median = 1.802).

Wilcoxon signed ranks - aqueous treatment data		N	Mean Rank	Sum of Ranks	Medians		Test statistics	
SMA2 - SMA1	Negative Ranks	60(a)	55.40	3,324.00	SMA1 median	0.788	Z(a)	-1.168
	Positive Ranks	48(b)	53.38	2,562.00	SMA2 median	0.813	Asymp. Sig. (2-tailed)	.243
	Ties	0(c)					Exact Sig. (2-tailed)	.245
	Total	108						
SMB2 - SMB1	Negative Ranks	58(d)	47.72	2,768.00	SMB1 median	0.390	Z(a)	-1.409
	Positive Ranks	39(e)	50.90	1,985.00	SMB2 median	0.399	Asymp. Sig. (2-tailed)	.159
	Ties	0(f)					Exact Sig. (2-tailed)	.160
	Total	97						
TYA2 - TYA1	Negative Ranks	50(g)	60.48	3,024.00	TYA1 median	1.313	Z(b)	-1.724
	Positive Ranks	71(h)	61.37	4,357.00	TYA2 median	1.362	Asymp. Sig. (2-tailed)	.085
	Ties	0(i)					Exact Sig. (2-tailed)	.085
	Total	121						
TYB2 - TYB1	Negative Ranks	48(j)	55.75	2,676.00	TYB1 median	1.816	Z(b)	-1.560
	Positive Ranks	65(k)	57.92	3,765.00	TYB2 median	1.839	Asymp. Sig. (2-tailed)	.119
	Ties	1(l)					Exact Sig. (2-tailed)	.119
	Total	114						
TYC2 - TYC1	Negative Ranks	46(m)	60.76	2,795.00	TYC1 median	1.082	Z(b)	-1.786

Wilcoxon signed ranks - aqueous treatment data		N	Mean Rank	Sum of Ranks	Medians		Test statistics	
	Positive Ranks	71(n)	57.86	4,108.00	TYC2 median	1.113	Asymp. Sig. (2-tailed)	.074
	Ties	0(o)					Exact Sig. (2-tailed)	.074
	Total	117						
TYD2 - TYD1	Negative Ranks	53(p)	54.36	2,881.00	TYD1 median	1.039	Z(b)	-0.668
	Positive Ranks	58(q)	57.50	3,335.00	TYD2 median	1.012	Asymp. Sig. (2-tailed)	.504
	Ties	0(r)					Exact Sig. (2-tailed)	.507
	Total	111						
TYE2 - TYE1	Negative Ranks	46(s)	54.41	2,503.00	TYE1 median	1.562	Z(b)	-2.056
	Positive Ranks	67(t)	58.78	3,938.00	TYE2 median	1.690	Asymp. Sig. (2-tailed)	.040
	Ties	0(u)					Exact Sig. (2-tailed)	.040
	Total	113						
a. SMA2 < SMA1; b. SMA2 > SMA1; c. SMA2 = SMA1; d. SMB2 < SMB1; e. SMB2 > SMB1; f. SMB2 = SMB1; g. TYA2 < TYA1; h. TYA2 > TYA1; i. TYA2 = TYA1; j. TYB2 < TYB1; k. TYB2 > TYB1; l. TYB2 = TYB1; m. TYC2 < TYC1; n. TYC2 > TYC1; o. TYC2 = TYC1; p. TYD2 < TYD1; q. TYD2 > TYD1; r. TYD2 = TYD1; s. TYE2 < TYE1; t. TYE2 > TYE1; u. TYE2 = TYE1							a. Based on positive ranks; b. Based on negative ranks;	

Table 20. Summary of Wilcoxon signed ranks test results for aqueous treatment samples, $p \leq .05$.

Wilcoxon signed ranks - organic solvent-based treatment data		N	Mean Rank	Sum of Ranks	Medians		Test statistics	
SMA2 - SMA1	Negative Ranks	69(a)	51.06	3,523.00	SMA1 median	0.881	Z(a)	-5.389
	Positive Ranks	23(b)	32.83	755.00	SMA2 median	0.844	Asymp. Sig. (2-tailed)	.000
	Ties	0(c)					Exact Sig. (2-tailed)	.000
	Total	92						
SMB2 - SMB1	Negative Ranks	67(d)	50.54	3,386.00	SMB1 median	0.395	Z(a)	-4.856
	Positive Ranks	25(e)	35.68	892.00	SMB2 median	0.381	Asymp. Sig. (2-tailed)	.000
	Ties	0(f)					Exact Sig. (2-tailed)	.000
	Total	92						
TYA2 - TYA1	Negative Ranks	38(g)	40.00	1,520.00	TYA1 median	1.612	Z(b)	-2.953
	Positive Ranks	58(h)	54.07	3,136.00	TYA2 median	1.629	Asymp. Sig. (2-tailed)	.003
	Ties	0(i)					Exact Sig. (2-tailed)	.003
	Total	96						
TYB2 - TYB1	Negative Ranks	36(j)	37.31	1,343.00	TYB1 median	2.315	Z(b)	-3.599
	Positive Ranks	60(k)	55.22	3,313.00	TYB2 median	2.390	Asymp. Sig. (2-tailed)	.000
	Ties	0(l)					Exact Sig. (2-tailed)	.000
	Total	96						
TYC2 - TYC1	Negative Ranks	31(m)	40.26	1,248.00	TYC1 median	1.949	Z(b)	-3.947
	Positive Ranks	65(n)	52.43	3,408.00	TYC2 median	2.040	Asymp. Sig. (2-tailed)	.000
	Ties	0(o)					Exact Sig. (2-tailed)	.000
	Total	96						
TYD2 - TYD1	Negative Ranks	39(p)	46.08	1,797.00	TYD1 median	1.802	Z(b)	-1.940
	Positive Ranks	57(q)	50.16	2,859.00	TYD2 median	1.827	Asymp. Sig. (2-tailed)	.052
	Ties	0(r)					Exact Sig. (2-tailed)	.052
	Total	96						
TYE2 - TYE1	Negative Ranks	32(s)	46.25	1,480.00	TYE1 median	3.687	Z(b)	-3.099
	Positive Ranks	64(t)	49.63	3,176.00	TYE2 median	3.835	Asymp. Sig. (2-tailed)	.002
	Ties	0(u)					Exact Sig. (2-tailed)	.002
	Total	96						
a. SMA2 < SMA1; b. SMA2 > SMA1; c. SMA2 = SMA1; d. SMB2 < SMB1; e. SMB2 > SMB1; f. SMB2 = SMB1; g. TYA2 < TYA1; h. TYA2 > TYA1; i. TYA2 = TYA1; j. TYB2 < TYB1; k. TYB2 > TYB1; l. TYB2 = TYB1; m. TYC2 < TYC1; n. TYC2 > TYC1; o. TYC2 = TYC1; p. TYD2 < TYD1; q. TYD2 > TYD1; r. TYD2 = TYD1; s. TYE2 < TYE1; t. TYE2 > TYE1; u. TYE2 = TYE1							a. Based on positive ranks; b. Based on negative ranks	

Table 21. Summary of Wilcoxon signed ranks test results for organic solvent-based treatment samples, $.01 \leq p < .001$, and $.001 \leq p < .000$.

The repeated aqueous treatment samples which were not frozen prior to sample processing and electrophoresis were also checked separately to see if freezing before processing caused any significant effect to the peak ratios. Four peaks exhibited a statistically significant change in peak height ratio. SMB1 (median = 0.234) was statistically significantly lower than SMB2

(median = 0.346), $T = 47$, $p = .030$ (exact significance); TYC2 (median = 0.827) was significantly higher than TYC1 (median = 0.786), $T = 90$, $p = .001$ (exact significance); TYD2 (median = 0.766) was significantly lower than TYD1 (median = 0.774), $T = 115$, $p = .026$ (exact significance); and TYE2 (median = 1.229) was significantly higher than TYE1 (median = 1.016), $T = 49$, $p = .000$ (exact significance). Although differences in peak height ratios between electrophoresis runs and data collection were found to be statistically significant in the samples that were processed and electrophoresed prior to freezing, the overall trend of higher median values in run 2 is largely consistent with the aqueous treatment data from those samples that were frozen prior to processing and electrophoresis (above). Again, although differences between runs were found to be statistically significant, the differences between medians were typically quite small (less than 0.1). See Table 22 for a summary of the statistical test results.

Wilcoxon signed ranks		N	Mean Rank	Sum of Ranks	Medians		Test statistics	
SMA2 - SMA1	Negative Ranks	15(a)	12.93	194.00	SMA1 median	0.510	Z(a)	-0.470
	Positive Ranks	11(b)	14.27	157.00	SMA2 median	0.513	Asymp. Sig. (2-tailed)	.638
	Ties	0(c)					Exact Sig. (2-tailed)	.653
	Total	26						
SMB2 - SMB1	Negative Ranks	16(d)	10.19	163.00	SMB1 median	0.234	Z(a)	-2.165
	Positive Ranks	4(e)	11.75	47.00	SMB2 median	0.346	Asymp. Sig. (2-tailed)	.030
	Ties	0(f)					Exact Sig. (2-tailed)	.030
	Total	20						
TYA2 - TYA1	Negative Ranks	15(g)	19.27	289.00	TYA1 median	1.084	Z(a)	-0.467
	Positive Ranks	17(h)	14.06	239.00	TYA2 median	1.057	Asymp. Sig. (2-tailed)	.640
	Ties	0(i)					Exact Sig. (2-tailed)	.651
	Total	32						
TYB2 - TYB1	Negative Ranks	13(j)	15.92	207.00	TYB1 median	1.418	Z(b)	-0.524
	Positive Ranks	17(k)	15.18	258.00	TYB2 median	1.475	Asymp. Sig. (2-tailed)	.600
	Ties	0(l)					Exact Sig. (2-tailed)	.612
	Total	30						
TYC2 - TYC1	Negative Ranks	7(m)	12.86	90.00	TYC1 median	0.786	Z(b)	-3.096
	Positive Ranks	24(n)	16.92	406.00	TYC2 median	0.827	Asymp. Sig. (2-tailed)	.002
	Ties	0(o)					Exact Sig. (2-tailed)	.001
	Total	31						
TYD2 - TYD1	Negative Ranks	9(p)	12.78	115.00	TYD1 median	0.774	Z(b)	-2.216
	Positive Ranks	20(q)	16.00	320.00	TYD2 median	0.766	Asymp. Sig. (2-tailed)	.027
	Ties	0(r)					Exact Sig. (2-tailed)	.026
	Total	29						
TYE2 - TYE1	Negative Ranks	7(s)	7.00	49.00	TYE1 median	1.016	Z(b)	-3.507
	Positive Ranks	21(t)	17.00	357.00	TYE2 median	1.229	Asymp. Sig. (2-tailed)	.000
	Ties	0(u)					Exact Sig. (2-tailed)	.000
	Total	28						
a. SMA2 < SMA1; b. SMA2 > SMA1; c. SMA2 = SMA1; d. SMB2 < SMB1; e. SMB2 > SMB1; f. SMB2 = SMB1; g. TYA2 < TYA1; h. TYA2 > TYA1; i. TYA2 = TYA1; j. TYB2 < TYB1; k. TYB2 > TYB1; l. TYB2 = TYB1; m. TYC2 < TYC1; n. TYC2 > TYC1; o. TYC2 = TYC1; p. TYD2 < TYD1; q. TYD2 > TYD1; r. TYD2 = TYD1; s. TYE2 < TYE1; t. TYE2 > TYE1; u. TYE2 = TYE1							a. Based on positive ranks; b. Based on negative ranks;	

Table 22. Summary of Wilcoxon signed ranks test results for repeated samples, run 1 not frozen before electrophoresis $.05 \leq p < .01$, $.01 \leq p < .001$, and $.001 \leq p < .000$.

To summarise the results of the Wilcoxon signed ranks test, although variation was found between runs that was statistically significant, these differences were typically quite small, less than 0.1, and would not have altered the outcome of this study. As all treatments were compared against the water control, changes between runs would effectively be equalised,

because the water control median would change in line with the changes observed in the treated samples. As peak height ratios overall seemed to increase in the second electrophoresis run, the only potential exaggerated effect would be for those treatments that tended to show a reduction in median peak height ratio values in the second run, which was observed in the SM peak height ratios of the organic solvent-based treatments. In these cases, as the water control value increased, but the treatment values decreased, thus potentially making these treatments seem more damaging than perhaps they truly were. As these median differences were quite small (SMA1 median – SMA2 median = 0.037, and SMB1 median – SMB2 median = 0.014), such small shifts in median would have been almost imperceptible in the scatterplots and boxplots. Differences between runs appear to have had little effect on the results, as the majority of treatments determined by this study to be safe are organic solvent-based.

5.2. Mummy case study

The effects on DNA of ancient Egyptian mummification methods, as understood to date, were examined and the sensitivity of the methods used reported. PCR amplification of 8 ancient Egyptian cat mummy hair samples failed. Recent rabbit mummy DNA was successfully amplified by PCR, and the PCR products were cloned and sequenced to assess DNA damage.

5.2.1. Quantification results

Purified PCR product concentration was determined to use in calculating the sensitivity of both the extraction procedure as well as the PCR primers and amplification conditions. The concentration of purified PCR products was determined as follows:

A best-fit line was plotted for the calibration standards using Excel 2003, and the sample values were calculated as:
Cat PCR product = 19.7400602 ng/mL
Rabbit PCR product = 7.50734588 ng/mL

However, the original concentration of the purified PCR products was 1000x this concentration as 1 µl of PCR product was added to 999 µl water), thus:
Cat PCR product = 19740.0602 ng/mL (=19740.0602 pg/µl or $1.97400602 \times 10^{-8}$ g/µl)
Rabbit PCR product = 7507.34588 ng/mL (=7507.34588 pg/µl or $7.50734588 \times 10^{-9}$ g/µl)

To determine the number of copies per µl, the following calculation was done:
(610 g/mol bp) x (1 mol/6.02×10^{23} bp) = 1.013×10^{-21} g/bp

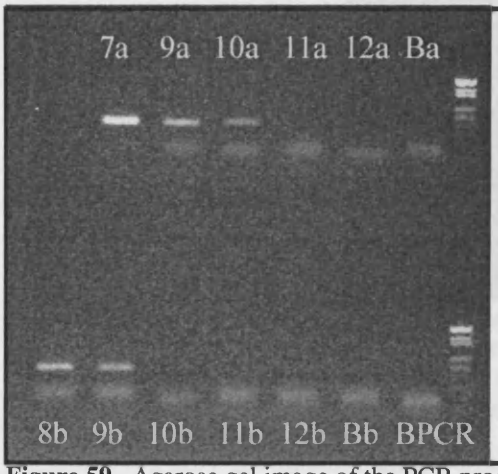
Cat PCR product consists of 164bp x 1.013×10^{-21} g/bp = 1.66132×10^{-19} g/molecule
($1.97400602 \times 10^{-8}$ g/µl) x (1/1.66132×10^{-19} g/molecule) = $1.18787647 \times 10^{11}$ copies/µl

Rabbit PCR product consists of 133bp x (1.013×10^{-21} g/bp) = $1.3476744 \times 10^{-19}$

($7.50734588 \times 10^{-9}$ g/µl) x ($1/1.3476744 \times 10^{-19}$ g/molecule) = 5.5705931×10^{10} copies/µl

5.2.2. Determination of extraction sensitivity

Using the series dilution of purified recent cat PCR products (as described in section 4.2.8), it was determined that the extraction technique and subsequent PCR was successful at endogenous DNA concentrations of between 114 and 1140 copies (samples 10a and 9b in Figure 59), and no contamination was identified in the extraction blanks or the second PCR blank.



Number of copies of DNA template per sample:

First extraction	Second extraction
7a = 114×10^3	8b = 114×10^2
9a = 114 x 10	9b = 114 x 10
10a = 114	10b = 114
11a = 11	11b = 11
12a = 1	12b = 1
Ba = extraction blank	Bb = extraction blank
	BPCR = PCR blank

The far right well contains a size standard.

Figure 59. Agarose gel image of the PCR products using the serial dilution solutions as samples to determine the sensitivity of the extraction technique.

5.2.3. Determination of amplification sensitivity

Using the series dilution of purified mummified rabbit and recent cat PCR products (as described in section 4.2.9), it was determined that rabbit_cytbF/R were PCR-sensitive to 6-59 copies of the DNA template (well 28 in Figure 60), and cat-cytbF/R were PCR-sensitive to 1-11 copies of the DNA template (well 12 in Figure 60).

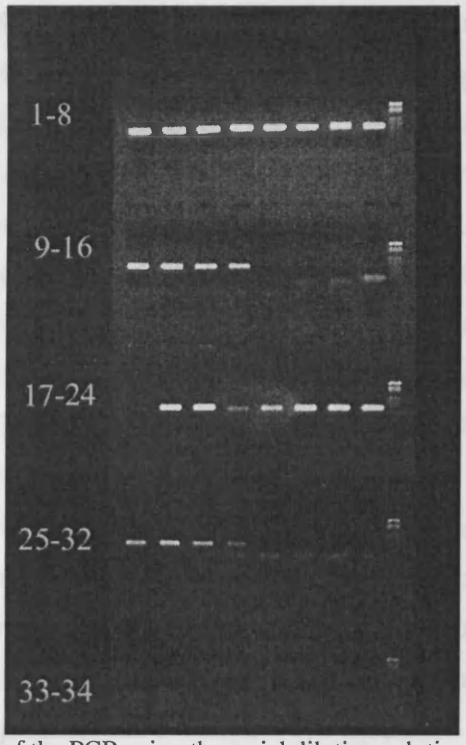


Figure 60. Agarose gel image of the PCR using the serial dilution solutions as template DNA to determine the sensitivity of the amplification conditions and primers. Wells 1-16 are the cat serial dilution samples with a starting concentration of $114x10^8$. Well 17 is a cat blank. Wells 18-33 are rabbit serial dilution samples with a starting concentration of $59x10^8$. Well 34 is a rabbit blank. The far right well contains a size standard.

None of the eight ancient Egyptian cat mummy hair samples yielded amplifiable DNA. Using the same extraction and PCR conditions, the recent cat sample yielded amplifiable DNA, indicating that the protocols followed were appropriate for the material, even if only traces of endogenous DNA survived, based on the calculations above. The recent rabbit mummy sample also yielded amplifiable DNA, indicating that the mummification process employed did not damage DNA beyond recovery using current extraction and amplification protocols.

Unfortunately, no conservation records exist for the ancient Egyptian mummies used in this study, although it was suggested by conservation staff that some consolidation with bone glue or shellac was possible in the past (Doyle 2006, pers. comm.). There was some evidence on some specimens of past insect activity, and although it was not possible to tell when any insect attacks took place, like most dry soft tissue/fur materials, it is possible that these specimens had been subjected to pesticides at some time. Therefore, it is not possible to determine if DNA in the samples taken were too degraded due to heat or other environmental factors, or if past conservation treatments caused damage or inhibited the extraction and amplification procedures.

5.2.4. Sequencing results

The rabbit mummy PCR products were cloned and sequenced to identify sequence changes that may be indicative of damage. Sequencher 4.1 software was used to process the sequences. Cloned sequences were compared to that of *Oryctolagus cuniculus* GenBank accession number

X54172 in the EMBL-EBI database (accessed 21/9/05). Of the 7 complete 133bp coding sequences recovered, only one base change at position 56 from an A to a G occurred in one sample from that of the published sequence (see Table 23). This base change is one of the most commonly observed, due to deamination of adenine resulting either from post-mortem diagenesis or from *Taq* polymerase transcription errors (Binladen et al. 2006). The relatively low rate of error suggests that little damage has occurred to the DNA in the rabbit hair in the 5 years after its death and subsequent mummification. This is significant, as it indicates that simple mummification techniques, consisting of an alcohol wash and multiple changes of natron, are not damaging to DNA in the short term. The inability to extract DNA from ancient Egyptian material in several reports (Höss et al. 1996; Marota et al. 2002), including Pääbo's (1985) initial account, as DNA from only one mummy out of 23 sampled was claimed to yield a cloned sequence, therefore must be due to other factors. It is thought that prolonged exposure to a relatively high average temperature may be responsible for extensive DNA damage (Gilbert et al. 2005b; Marota et al. 2002). It is also possible that different mummification methods used other compounds or that preparation and/or conservation treatments were administered that could inhibit PCR or cause DNA damage or cross-linking, but further research into these possibilities is required.

Specimen	Sequence
Rabbit_X54172	GAATCCTCGTCGCAGATCTTCTCACACTCACATGAATCGGAGGCCAACCAGTAGAACACCCGTTCATCACCATTGGACAAGTAGCATCTGTCCTCTACTTCACCACCATCCTTATTCTTATACCCCTCGCAAG
1_66_F01_11	GAATCCTCGTCGCAGATCTTCTCACACTCACATGAATCGGAGGCCAACCAGTAGAACACCCGTTCATCACCATTGGACAAGTAGCATCTGTCCTCTACTTCACCACCATCCTTATTCTCATACCCCTCGCAAG
1_66_F02_12	GAATCCTCGTCGCAGATCTTCTCACACTCACATGAATCGGAGGCCAACCAGTAGAACACCCGTTCATCACCATTGGACAAGTAGCATCTGTCCTCTACTTCACCACCATCCTTATTCTCATACCCCTCGCAAG
1_66_E01_09	GAATCCTCGTCGCAGATCTTCTCACACTCACATGAATCGGAGGCCAACCAGTAGAACACCCGTTCATCACCATTGGACAAGTAGCATCTGTCCTCTACTTCACCACCATCCTTATTCTCATACCCCTCGCAAG
1_66_C01_05	GAATCCTCGTCGCAGATCTTCTCACACTCACATGAATCGGAGGCCAACCAGTAGAACACCCGTTCATCACCATTGGACAAGTAGCATCTGTCCTCTACTTCACCACCATCCTTATTCTCATACCCCTCGCAAG
1_66_D01_07	GAATCCTCGTCGCAGATCTTCTCACACTCACATGAATCGGAGGCCAACCAGTAGAACACCCGTTCATCACCATTGGACAAGTAGCATCTGTCCTCTACTTCACCACCATCCTTATTCTCATACCCCTCGCAAG
1_66_G02_10	GAATCCTCGTCGCAGATCTTCTCACACTCACATGAATCGGAGGCCAACCAGTAGAACACCCGTTCATCACCATTGGACAAGTAGCATCTGTCCTCTACTTCACCACCATCCTTATTCTCATACCCCTCGCAAG
1_66_E02_14	GAATCCTCGTCGCAGATCTTCTCACACTCACATGAATCGGAGGCCAACCAGTAGAGCACCCGTTCATCACCATTGGACAAGTAGCATCTGTCCTCTACTTCACCACCATCCTTATTCTCATACCCCTCGCAAG

Table 23. Sequence alignment of published rabbit sequence (Rabbit_X54172) and sequences obtained from 7 cloned colonies from the recent rabbit specimen. The single base change identified in one of the recent rabbit mummy clones is highlighted in the last sequence.

Chapter 6. Discussion

Several research questions were posed at the beginning of this project regarding the effects of preparation and conservation treatments on DNA, and a four part examination of the subject was undertaken, consisting of assessing previous related research, identifying treatments used in the past, developing a screening test to assess the effects of treatments on short strands of DNA *in vitro*, and investigating the effects of ancient Egyptian mummification techniques on DNA. Throughout the course of this study, a number of other pertinent issues were discovered and explored. In addition to presenting the answers to the research questions, other relevant issues are also discussed here, including: the future utility of the method developed in this study, associated problems discovered with the field of conservation and the preservation of biomolecules, as well as considerations for best practice in conservation regarding DNA preservation and suggestions for future research.

6.1. Results and answers to research questions

6.1.1. What effect, if any, do preparation and conservation treatments have on DNA?

The method developed for this study enabled the assessment of damage, in the form of strand breakage, to two short fragments of DNA *in vitro* as the result of exposure to preparation and conservation treatments. DNA damage was identified as a reduction in DNA peak height when the samples were run on an ABI PRISM® 3100 Genetic Analyzer. The preservation of the DNA fragments in treated samples was assessed relative to the preservation of the same fragments in the control samples. The degree of damage to each fragment was not identifiable (i.e. whether the fragments were sheared in one or many places), but did not appear to be systematic in any way, as no additional obvious peaks were seen in the electropherograms. Various methods of assessment were employed, including visual inspection of electropherograms, semi-quantitative assessment of damage ratios in boxplots and scatterplots, as well as calculation of the percentage of DNA preserved by each treatment (as an average for all samples of each treatment), and statistical analysis using the Mann-Whitney and Wilcoxon signed ranks tests.

A conservative approach was taken in determining which treatments are either safe or unsafe to use based on their effects on DNA. Any treatment administered under test conditions giving a damage ratio less than the lower standard deviation of the control for either of the DNA fragments tested was deemed unsafe (i.e. equating to less than 80% DNA preservation), and any treatments with a damage ratio greater than the lower standard deviation (i.e. greater than 80 % DNA preservation) were deemed safe (see Table 24 for a final list). Using these criteria, the majority of treatments tested were deemed damaging to some degree. All treatments heated to 37°C or higher were found to be damaging. Also, all aqueous treatments were found to be

damaging, with the exception of arsenic trioxide and sodium chloride, which seemed to buffer the damaging effects of water. Some evidence was found suggesting that damage resulting from different chemical treatments was sequence specific, and that DNA fragments with a higher proportion of GC bonds were more resistant to damage. Of the two fragments used in this study, TAT consisted of 36% GC bonds, and SRY 4064 consisted of 47% GC bonds. The final list of treatments deemed either safe or unsafe based on the results of this study is largely based on the results for the TAT fragment, which provided the most conservative list, as with the exception of heating water to 37°C for 48 hours, all treatments deemed safe for the TAT fragment were also safe for the SRY 4064 fragments (as heating water to 37°C was found to be damaging to the SRY 4064 fragment, it was deemed unsafe in the final list). Of those treatments deemed unsafe for use, acetic acid, mineral oil, oxalic acid, pepsin, sodium hypochlorite, sodium sulfide, turpentine and heating to 80°C or higher were the treatments most damaging to DNA. Other treatments on the unsafe list caused moderate damage, but it is unknown if the degree of damage would be sufficient to prohibit use of treated material for amplification by PCR. However, from a conservation point of view, the fact that damage was sustained by DNA as a result of these treatments makes them inappropriate for use, and alternative treatments should be sought. Of those treatments deemed safe to use, some evidence was found that amyl acetate, IMS, sodium chloride and xylene may have a positively preserving effect, but the effects were not consistent for both DNA fragments, so further research into treatments that may enhance DNA preservation is required.

Treatments considered safe	Treatments considered unsafe
1:1 acetone:IMS	Any treatment requiring heating to 37°C or higher
1:1 ethanol:ether	Acetic acid
Acetone	Acrylic emulsion
Amyl acetate	Alum
Arsenic trioxide	Ammonium hydroxide
Chloroform	Benzene
Ethyl acetate	Carbon tetrachloride
IMS	Cellulose nitrate [in 1:1 ethanol:ether]
Sodium chloride	Detergent
Toluene	EDTA
Trichloroethylene	Ethanol
Xylene	Gasoline [in ethanol/turpentine]
	Gum arabic
	Hydrogen peroxide
	Kerosene
	Linseed oil [in turpentine]
	Mercury (II) chloride [in ethanol]
	Methylmethacrylate/ethylacrylate [in acetone]
	Mineral oil
	Oxalic acid
	Pepsin
	Poly(vinyl) butyral resin [in 1:1 acetone:IMS]
	Potassium carbonate
	PVAC
	PVAC/PVAL
	Shellac [in ethanol]
	Sodium bicarbonate
	Sodium carbonate
	Sodium chloride - 24H @ 37°C
	Sodium hydroxide
	Sodium hypochlorite

Treatments considered safe	Treatments considered unsafe
	Sodium perborate
	Sodium sulfide [in saline]
	Turpentine
	White spirit

Table 24. List of treatments safe to use and unsafe to use based on their effects on DNA *in vitro* as found in this study.

Incorporating the results from this study and other published results (Kigawa et al. 2003; Williams 1999) into the list predicting the effects of treatments on DNA compiled by Brown (1999), demonstrates that more treatments are probably unsafe for DNA than are safe for DNA (see Table 25); quite the opposite result from the original list published (see Table 2). Many of these treatments have been and still are quite commonly used, so the scope of potential damage resulting from preparation and conservation treatments administered within collections may be substantial.

Unsafe (induce chemical modifications to DNA under mild conditions)	Probably unsafe	Probably safe
Aldehydes	1,1,1,2 tetrafluoroethane[2]	1:1 acetone:IMS[3]
Formaldehyde	Acetic acid[3]	1:1 ethanol:ether[3]
Glyoxal	Acrylic emulsion[3]	Acetone
Ninhydrin	Alum (aluminium ammonium sulfate)[1]	Alcohols
Alkylating agents	Alum (potassium aluminium sulfate dodecahydrate)[3]	~~Alum~~[1]
Alkyl halides		Amyl acetate[3]
Dimethyl sulphate	Ammonium hydroxide	~~Arsenicals~~[1], (arsenic trioxide)[3]
Ammonium derivatives	Arsenicals (arsenic trioxide)[1]	Bendiocarb
Hydrazine	Benzene[3]	~~Benzene~~[3]
Hydroxylamine	Carbon tetrachloride	Borax
Semicarbazide	Cellulose nitrate[3]	Camphor
Aromatic nitrogen compounds	Chloropicrin	Carbolic acid (phenol)
Aromatic amines	Chromic acid	Carbon disulphide
Azo-dyes	Citric acid	Chloroform
Bisulphites	Detergent (enzyme active)[3]	Chromates
Sodium bisulphite	Dichlorvos	Dioxane
Borohydrides	EDTA[3]	Ether
Sodium borohydride	Ethanol[3]	Ethyl acetate[3]
Carbodiimides	Ethylene dichloride	~~Ethylene oxide~~[2]
N,N-dicyclohexylcarbodiimide	Ethylene oxide[2]	Gasoline
Halogens	Gasoline[3]	Glycerine
Bromine	Glutaraldehyde	Glycerol
Iodine	Gum arabic[3]	Hydrogen cyanide
Iodine chloride	Kerosene[3]	IMS[3]
Mercurics	Lead salts	Lysol
Mercury (II) acetate	Lindane	~~Magnesium carbonate~~[1]
Mercury (II) chloride	Linseed oil[3]	~~Naphtha (white spirit)~~[3]
Nitrites	Magnesium carbonate[1]	Naphthalene
Nitrous acid	Mercuric salts	Phosphine
Sodium nitrite	Methyl bromide	Potash
N-Nitroso compounds	Methyl iodide[2]	Potassium nitrite
Nitrosourea	Methylmethacrylate/ethacrylate[3]	Potassium phosphate
Oxidizing agents	Mineral oil[3]	Propoxur
Hydrogen peroxide	Organomercuric salts	~~Salt (sodium chloride)~~[1] (sodium chloride)[3]
Osmium tetroxide	Organophosphates	Sodium acetate
Peracids	Oxalic acid[3]	~~Sodium bicarbonate~~[3]
Potassium permanganate	Paradichlorobenzene	Sodium dithionite
	Pepsin[3]	Sodium phosphate

Unsafe (induce chemical modifications to DNA under mild conditions)	Probably unsafe	Probably safe
	Pentachlorophenol	Toluene[3]
	Perchloroethylene	Trichloroethylene[3]
	Poly(vinyl) butyral resin[3]	~~Turpentine~~[3]
	Potassium carbonate[3]	Xylene[3]
	Potassium nitrate[1]	
	Propylene oxide[2]	
	PVAC[3]	
	PVAC/PVAL[3]	
	Salt (sodium chloride)[1]	
	Shellac [in ethanol][3]	
	Sodium bicarbonate[3]	
	Sodium borate[1]	
	Sodium carbonate[3]	
	Sodium chloride – 24H @37°C[3]	
	Sodium hydroxide[3]	
	Sodium hypochlorite[3]	
	Sodium perborate[3]	
	Sodium silicofluoride	
	Sodium sulfide[3]	
	Sulphur fluoride	
	Turpentine[3]	
	White spirit[3]	
	Heating to 37°C or higher	

Table 25. Revised version of Brown's (1999) list predicting the effects of treatments on DNA, updated to include experimental results. Compounds struck-through represent cases where initial predictions do not agree with experimental results, and superscript numbers indicate the source of the results as follows: 1 = (Williams 1999), 2 = (Kigawa et al. 2003), and 3 = results of this study.

Some inconsistencies were discovered when comparing the results of this study to previous research, but this is most likely due to the fact that treatments were administered to DNA *in vitro*, without the benefit of any buffering effects that would most likely be provided to DNA by hard and/or soft tissues. Therefore, damage to DNA observed in this study was probably greater than in the skin and muscle tissue samples used in the other published reports. Further research is required to confirm the information in Table 25, particularly regarding the effects of those treatments not experimentally tested thus far, and to investigate the effects of treatments on DNA in different tissue types.

6.1.2. Based on these results, is it possible to predict the viability of DNA research using material treated in the past?

The viability of collection material is difficult to predict, primarily because of the lack of detailed treatment documentation both in the preparation and conservation literature as well as within museum records – it is almost impossible to know the full treatment history of any specimen in an existing collection. This was consistently found to be a problem during the literature review, where many instructions for treatments were insufficiently documented so that few treatments could be reliably replicated. Lack of treatment documentation was also encountered in several different museum collections when attempting to locate specimens suitable for use as case studies to investigate the effects of treatments carried out in the past, and/or the effects of different storage environments on collection materials from the same

specimen. In order for such a case study to be useful, the full treatment history of a specimen must be well documented, so that any damage sustained could potentially be attributed to a specific cause. The inability to account for all potential treatments carried out as routine "housekeeping", such as periodic cleaning or pest control measures, precluded the use of most collection materials in this study, as little value would be gained by simply saying that DNA in a particular specimen was damaged without being able to identify the source of the damage.

A further difficulty in determining the viability of DNA research on specimens from collections is that aside from freshly collected tissues, it is impossible to account for the state of DNA preservation in a specimen when it entered a collection. DNA damage may result not only from treatments administered in the museum context, but also the environmental conditions to which specimens were exposed prior to materials coming into collections. This can be particularly problematic for unprovenanced archaeological materials, as without knowledge of the site from which an object has been recovered, it is difficult to take into account the potential effects of the burial environment on DNA preservation.

The mummy case study illustrates all of these issues. The rabbit cytochrome B sequence targeted in the mummy case study had a similar GC content (49%) to the SRY 4064 fragment (47%) used in the screening test, and would be expected to respond to treatments similarly. Ethanol, sodium carbonate, sodium bicarbonate and sodium chloride were all found to be safe or potentially safe for the SRY 4064 fragment in the screening test, so it would be expected that mummification using an alcohol wash and desiccation by natron (a combination of evaporitic salts including sodium carbonates and sodium chloride) would not cause extensive damage to the rabbit sequence studied, as was found to be the case. The cat cytochrome B sequence targeted in the mummy case study had a slightly lower GC content (43%), and would therefore be expected to be only slightly less well preserved than the SRY 4064 fragment. However, it was not possible to extract and PCR amplify this sequence from any of the 8 mummies sampled. This may be due to many factors, including additional unidentified compounds used in the mummification procedure or in conservation treatments administered either before or after the mummies were placed in the collection. As the mummies are unprovenanced, little can be surmised about the effects the burial environment may have had on DNA preservation, but it is thought that long-term exposure to elevated average temperatures has damaged DNA in ancient Egyptian specimens that have not yielded PCR amplifiable DNA (Gilbert et al. 2005b; Marota et al. 2002).

Only when the full storage and treatment history of a specimen is known can the results of the screening test from this study be used as a guide to determine which specimens may be more or less suited for DNA anslyses. Based on the results of this study, it is suggested that specimens

exposed to those treatments deemed to be most damaging (acetic acid, mineral oil, oxalic acid, pepsin, sodium hypochlorite, sodium sulfide, turpentine and heating to 80°C or higher) would be less favoured when selecting specimens for DNA samples. Other treatments deemed to be unsafe may not cause extensive damage, and depending on the state of DNA preservation prior to treatment, the tissue sampled, the target sequence, and the efficiency of DNA extraction and amplification methods, such treated materials may yield DNA suitable for research. Specimens that have never been treated or have only been exposed to those treatments deemed to be safe would be preferred candidates for sampling.

As damage may be sequence specific, variable success may be had with DNA samples from specimens depending on the target sequence. To increase the likelihood of PCR amplification, it is suggested that primers are designed to amplify sequences with high GC content whenever possible, as such sequences should be more resistant to damage resulting from many of the chemicals commonly used in preparation and conservation treatments, as found in this study.

6.1.3. Is it possible to suggest materials that are preferred for use based upon minimal effect, or possibly even promoting DNA preservation?

As most of treatments tested in this study were found to be damaging to DNA, only a few suggestions can be made about methods and materials suitable for DNA preservation, many of which are standard conservation guidelines, but are worth reiterating. Perhaps the most important generalisation to be made is that mechanical treatments and preventive conservation measures are preferred to chemical treatments to promote DNA preservation. As the potential effects of chemical treatments on DNA extraction, PCR amplification and other analyses are still unknown, the availability of untreated specimens is the best way to enhance the biochemical research potential of specimens in collections. If it is not possible to clean a specimen mechanically (e.g. dry brushing or removing encrustations with a scalpel), the collection of samples prior to treatment should be considered. Leaving some elements of a specimen untreated or using different treatments on different elements of the same specimen might also warrant consideration, to spread the risk of aged treatment residues affecting DNA preservation and future analyses.

Eight of the ten treatments deemed to be safe for use were organic solvents. Although organic solvent-based treatments have become a second choice for many conservators on health and safety grounds, the use of less hazardous organic solvent-based treatments with the appropriate precautions taken may be preferred for materials requiring treatment and likely to be sampled for DNA. For example, acetone and IMS should be considered for use rather than benzene or carbon tetrachloride, as acetone and IMS are not only less hazardous to living people, they are less damaging to DNA in specimens.

Although the effects of low temperatures were not tested in this study, damage to DNA was found to increase with temperature. Low temperatures have been found to enhance DNA preservation both in archaeological contexts (Höss et al. 1996; Lindahl 1993) and storage environments (Benecke 2005; Dessauer et al. 1990). Although extreme low temperature storage may not be appropriate for collection materials due to their mechanical properties or the risk of condensation, low temperature storage of samples should be considered. Furthermore, freezing for pest control is preferred over the use of high temperatures or microwaves (or chemical treatment) whenever possible.

Both acetic acid and oxalic acid were found to be extremely damaging to DNA during this study, supporting the assertion that acidic conditions damage DNA (Lindahl 1993). Use of acidic treatments to remove carbonate encrustations or for other purposes should therefore not be used on sub-fossil material that may yield DNA. It can also be assumed that acidic storage materials could be damaging to DNA and should therefore be avoided.

A broader discussion of conservation best practice for DNA preservation can be found in Section 6.3.

6.2. Associated problems identified

Throughout the course of this research, a number of issues were found that relate to the conservation profession and the interaction between conservators and researchers. Rose (1991) identified two activities requiring urgent attention in conservation: improved documentation procedures and research for improved treatment development, particularly with regard to biochemical preservation. It is disappointing to see that 15 years later, these remain fundamental problems.

It is known that many museum collections (Rose 1991; Stroz et al. 1993; Williams 1999) and archaeological fieldwork reports (Caldararo 1987) lack complete and accurate preparation and conservation records. All of the major codes of ethics and codes of practice guiding conservation professionals (American Institute for Conservation of Historic & Artistic Works 1994; European Confederation of Conservator-Restorers Organisations 2002), include thorough and transparent documentation as an element of ethical practice. However, such guidelines are typically quite general, for example stating a conservator should "document examination, scientific investigation, and treatment by creating permanent records and reports" (American Institute for Conservation of Historic & Artistic Works 1994). Typically, practical guidance as to what should be recorded and how such documentation should be stored and made accessible is not detailed. Although nothing can be done to rectify the situation retrospectively, greater care must be taken to ensure this problem does not continue. With an increase in biochemical testing methods used on museum collections, accurate records may be an invaluable resource

for making decisions on sampling and viable research within collections.

Throughout the course of this study, the non-specific documentation of chemicals used in conservation treatments was consistently found to be problematic. Common names change over time and vary by location (see Table 4). Additionally, some common names may refer to a variety of chemical compounds, for example alum may be understood to mean aluminium potassium sulfate, aluminium ammonium sulfate, as well as other compounds and EDTA may refer to a disodium, trisodium or tetrasodium salt, amongst other varieties. Some compounds may be available in both anhydrous and hydrous forms, which should be specified whenever used. The use of ambiguous terms can lead to confusion or errors when attempting to replicate treatments, therefore it is essential to use chemical names, formulas or other identifiers such as CAS numbers when documenting conservation treatments.

Another problem identified during the literature review for this study was the use of unclear measurements or concentrations. When documenting chemicals in treatments, measurements should be provided as well as concentrations to eliminate any confusion. This is particularly important for some chemicals where various concentrations of a particular compound may be available on the market. References using solutions containing chemicals such as hydrogen peroxide, sodium hypochlorite and ammonia solutions routinely omitted essential details such as the percent volume of hydrogen peroxide, the percentage of available chlorine in sodium hypochlorite or the percent NH_3 of the original ammonia stock solutions. Providing the volume of stock solution used as well as specific details of the stock solution is the only way to provide complete and accurate references to concentrations in documentation.

Much research has been undertaken on the viability of collections material for research purposes with little regard given to the role of the methods and materials used by conservators to care for collections (Caldararo and Gabow 2000). There is a need for greater awareness by conservators about the specialised knowledge they have of their materials and methods and the effects their use may have on research results. Although little may currently be known about the effects of specific treatments on some analyses, conservators should be involved in actively researching this subject. Greater collaboration is required between conservators and researchers, and conservators must also play a more central role in advising on sampling strategies. Conservators are in a unique position to improve the quality of research based on collections materials both by improving the conditions under which collections are cared for and by using their knowledge of the materials sciences to assist researchers in sampling and devising research strategies – not to be involved in this process would be irresponsible.

Although the use of commercial proprietary products has been criticised in the archaeological

and palaeontological literature (Howie 1995; Shelton and Johnson 1995), there is an alarming trend in the use of proprietary products in the field biology and forensics professions. Routinely citing problems with running out of or difficulty in sourcing pure solvents for specimen preservation during fieldwork, as well as impromptu specimen collection, field biologists have suggested the use of acetone-based nail varnish remover (NVR) or hard liquors like gin (Lee and Beynon 2004) or vodka (Oakenfull 1994). Admitting that cold storage in liquid nitrogen or dry ice is the best method for preserving specimens collected with DNA analysis in mind, Lee and Benyon concluded that "in situations where laboratory chemicals are not immediately available, NVR is a suitable material for the short-term preservation of samples intended for use in PCR analyses" (2004: 749). However, only one brand of NVR was tested in this study, and the percentage of acetone and other compounds it contained was not mentioned; these products are rarely pure acetone and may contain a variety of different chemicals, such as N-butyl acetate, isopropyl alcohol, methyl ethyl ketone, gelatin (which could contain contaminating DNA) and other unspecified perfumes and colorants as well as an array of impurities which were not considered in their tests. The use of such compounds may be acceptable in emergencies and for the short-term in situations when multiple specimens can be collected for analyses, but the use of proprietary products for processing more valuable specimens is questionable.

The use of proprietary products to process human remains forensic evidence is worrying. Preservation of the "chain of evidence", or maintaining the association and integrity (defined as a lack of tampering or contamination) of evidentiary materials, is stressed throughout the forensics literature (Benecke 2005; Lee and Ladd 2001), but little guidance is available regarding the preservation of DNA (Smith and Morin 2005). Similar to the situation in museums, early forensic analysis of bones and teeth was heavily reliant on morphological analysis, and the methods for processing remains were developed with this in mind. In spite of acknowledging the superior results obtained by using cold/warm water maceration or dermestids to process skeletal material, forensic practitioners developed alternative methods to simplify the defleshing process and produce results in a shorter time (Snyder et al. 1975; Stephens 1979). These initial methods consisted of two or three steps, including soaking material in hot chemical solutions to speed up tissue removal. Although both Snyder et al. (1975) and Stephens (1979) used relatively pure chemical compounds mixed in the laboratory, at around the same time the use of proprietary products, particularly dishwashing and laundry detergents, was being promoted. Stephens (1979) reported the use of Biz® in 1978, and as late as 2003 simmering remains in a laundry detergent/sodium carbonate solution followed by degreasing in ammonia and a final soak in Vinac dissolved in methanol to coat the bones was recommended as a routine method to prepare human remains, because it was fast, safe, cheap and used easily obtainable materials (Fenton et al. 2003). The following year studies assessing

the effects of forensic skeletal processing techniques on DNA began to appear. As the forensic identification of human remains began to be less dependent on traditional morphological analyses, and more subject to DNA analyses, a number of studies have been published which have identified that the methods most commonly used to prepare skeletal and dental materials are in fact damaging to DNA to varying degrees (Arismendi et al. 2004; Rennick et al. 2005; Steadman et al. 2006). With the awareness that DNA tests may need to be carried out some time after processing, the long-term stability of DNA in remains is important. It is difficult to predict the effects of proprietary products on remains over time or the effects of impurities on future analyses, and undisclosed changes to the formulations of products may have unanticipated effects. It would seem that there is a need for the principles of conservation to be incorporated into forensics training, and more research is required to find better methods to prepare evidentiary materials in order to best preserve DNA, particularly considering the importance of complete and accurate forensic analyses and their potential consequences.

6.3. Conservation considerations for best practice

Several publications regarding ancient DNA requirements have specifically targeted archaeologists (Brown 1998; Brown and Brown 1992; Thuesen 1995), however little has been done to inform conservators about the materials and methods used in DNA research and how the activities of conservators may affect such research. It has been noted that "[t]hough latecomers in the field, museums are in a particularly significant position to lead the way in best practices of genetic resources management, because of their experience in curating traditional collections" (Corthals and Desalle 2005: 819). It is likely that the establishment of facilities expressly dedicated to the preservation of biomolecules will continue to increase and that conservators should be at the forefront of the movement, but few publications have addressed this audience. Various pertinent suggestions can be found scattered throughout the literature, although this is in no way intended as an exhaustive conservation strategy for biomolecules, this is an attempt to briefly summarise issues of relevance both from the literature and the results of this study that may be useful in moving towards establishing policies for the management of collections with the maintenance of DNA in mind, which is of relevance to both conservators and collection managers. It should also be noted that different biomolecules and tissues will require different conservation considerations, and the necessary policies and procedures for preserving the biochemical content of specimens will likely vary by collection type.

Several publications have suggested various considerations and criteria to assess the merits of requests for samples for DNA research (DeGusta and White 1996; Pääbo et al. 1992), as well as the authenticity of reported results (Cooper and Poinar 2000; Gilbert et al. 2005a; Poinar 2003), which may be of use in determining sampling policies, but these will not be further discussed here.

6.3.1. Treatment

The effects of preparation and conservation treatments on DNA can be largely classed into three categories: contamination, inhibition and damage. Treatments may be the source either of DNA contamination inherent to the treatment, such as bone glue or fish glue which will contain DNA from the animals used to produce these materials (Cooper 1994; Nicholson et al. 2002), or of human DNA originating either from manufacturing processes or use. Application of treatments containing DNA will add exogenous DNA to a specimen, which may interfere with analyses depending on the species in question, the research design and protocols used for the study. Chemicals used in treatments may also inhibit the ability to extract DNA from collection materials or to use extracted DNA in a specific analysis such as PCR (Hall et al. 1992). Although some methods have been devised to deal with the inhibitory effects of some chemicals for specific analytical methods, identifying the difference between inhibition and DNA damage may not always be straightforward – more research into this subject is required to ensure optimal research output is gained from any specimen sampled. Chemicals present in treatments may cause damage to DNA through a variety of mechanisms that are yet not well understood, but may include denaturation, strand breakage, base modification and cross-linking (Lindahl 1993). Only recently has it been recognised that museum prepared specimens of relatively recent age yielded sequences with greater variability than archaeological specimens (Binladen et al. 2006). As DNA damage remains a limiting factor in the success of DNA studies, minimising the damage to collection materials is of utmost concern for conservators.

To avoid DNA contamination, it is standard practice in DNA research to use sterile water and other DNA free reagents. Although this may be impractical for conservators, some concessions should be made to minimise the potential impact of cross-specimen contamination. Stock solutions in which a specimen is immersed for treatment should not be re-used for other specimens, as this could lead to the accumulation and transference of contaminant DNA (Cooper et al. 1994). This also applies to water used for washing specimens (Gilbert et al. 2005c). Similarly, abrasives used for sandblasting specimens should not be recycled (Hagelberg 1994). Although it is unlikely that conservators will adopt extreme measures to prevent DNA contamination, such as the use of masks, gloves and other protective wear on a daily basis, such steps may be advisable when working with newly collected material (i.e. uncontaminated material) or during sampling procedures (see section 6.3.3).

If fieldwork is to be undertaken with the objective of collecting specimens for DNA analysis, any reagents, fixatives or other preservatives that are anticipated for use should be thoroughly tested prior to going into the field to ensure their compatibility with subsequent analytical methods. Pilot projects should be designed to test the effects of any treatments to be administered on the ability to extract viable DNA, at the very least, as well as on any additional

anticipated analytical methods.

Several "blank" samples are typically included in any DNA analysis procedure to test for contaminating DNA in reagents or labware. It may be useful in cases where conservation treatment is required for morphological examination of specimens that will also be subjected to DNA analysis, to make available samples of treatment solutions for use in PCR to test for contaminating DNA and/or inhibiting effects.

The benefits of a centralised documentation system when dealing with research requests cannot be underestimated. Full documentation of any treatment administered to a specimen should be maintained with the accession record. Publications containing results from tests done on specimens from a collection should similarly be kept with the accession record. By centrally storing copies of all documentation for a specimen, decisions may be more readily reached regarding the viability of specimen use for specific research questions. Furthermore, any samples taken from specimens should be fully cross-referenced (for sample documentation guidelines, see Dessauer et al. 1990, 1996).

Conservators need to play an active role in assessing the suitability of past and current treatments for new research objectives. Compliance with the conservation principle of minimal intervention is particularly important, as we are often unaware of the potential effects treatments may have on the future utility of collection material. Treatments should be devised to have the minimal effect on the broadest range of applications. In some instances it may be advisable to use multiple different treatments to address a single preservation problem, with the hope that at least one treatment will be compatible with future research objectives. The development of treatments expressly to enhance DNA preservation has also been called for (Poinar 2002). More experimental work should be undertaken with such issues in mind, so that published assessments of the effects of conservation treatments can improve upon the largely anecdotal body of evidence that currently exists. Complete documentation and publication of results obtained from treated materials will ensure that we can expand our knowledge on the effects of preparation and conservation treatments in the future.

6.3.2. Storage

It is generally accepted that cold and/or dry conditions facilitate DNA preservation in both archaeological contexts (Höss et al. 1996; Lindahl 1993) and storage environments (Benecke 2005; Cann et al. 1993; Dessauer et al. 1990, 1996). When designing storage for hard or soft tissue specimens, it may be beneficial to strive for lower temperature and relative humidity targets suitable for the mechanical properties of collection materials. Special care should be given to avoid conditions that encourage the growth of mould and fungi, which contribute contaminating DNA to specimens (Hagelberg 1994).

Various suggestions have been made as to the role museums should play in storing and processing DNA extracted from collections materials. International funding agencies have been asked to make DNA banking programmes a priority, as there is a lack of facilities available internationally to preserve DNA extracts (Savolainen and Reeves 2004). The establishment of facilities equipped to carry out high-throughput DNA research complete with robotic retrieval storage systems have been recommended for major museums, which could provide their services to other institutions (Tautz et al. 2003). Smaller museums have also been encouraged to develop ultracold storage facilities to maintain frozen tissue and extract collections (Cooper 1994), or to arrange for storage in established regional or national biomaterial archival facilities as a means of "backing-up" collections (Hanner et al. 2005). Several publications recommend returning aliquots of extracted DNA to the loan institution for storage and use in future research (Cann et al. 1993; Cooper 1994; Pääbo et al. 1992).

Although liquid nitrogen storage is considered superior to ultracold freezers, because it is not subject to power outages or mechanical failure (Corthals and Desalle 2005) and they maintain samples at a lower temperature, -196°C rather than -70 to -150°C, thereby better preserving molecular integrity and quality (Dessauer et al. 1990, 1996), due to the technicalities involved in maintaining liquid nitrogen stores and accessibility issues, ultracold freezers are more commonly used for frozen collection storage (Hanner et al. 2005). However, frost-free models should not be used, as DNA can degrade during defrosting cycles (Dessauer et al. 1990, 1996). As with any other type of collection, it is also important to have an emergency plan in place. In addition to establishing a monitoring routine and placing contact details on freezers in case of failure (Dessauer et al. 1990, 1996), an emergency plan should also include steps to enable optimal salvage efforts in the event of power or freezer failure, as plasma, red cells and muscle tissues may retain some viable proteins and DNA fragments, and some tissues may be valuable for studies aside from those intended when originally collected (Hanner et al. 2005). It is also necessary to establish a catalogue or database inventory to allow samples to be located as quickly as possible to minimise temperature fluctuations within cold storage units (Cato and Schmidly 1991; Corthals and Desalle 2005; Dessauer et al. 1990, 1996; Ioannou 2000). The benefits of maintaining a centralised, secure, well-organised and accessible cryogenic store are well presented by Corthals and Desalle (2005).

In addition to storing frozen extracts of DNA, it has more recently been suggested that extracted DNA can be stored at room temperature in a couple of ways. Owens and Szalanski (2005) have proposed applying extracted DNA to filter paper and storing it at room temperature, which is appealing for its ease of storage and distribution to researchers. Smith and Morin (2005) have suggested storing DNA at room temperature in trehalose, a compound found in organisms that

undergo periods of desiccation during their life cycles, after initial experimental trials. However, the long-term utility of both methods remains unknown.

6.3.3. Sampling

Problems associated with modern human contamination affecting ancient DNA studies are well known (Gilbert et al. 2005c; Malmström et al. 2005; Richards et al. 1993; Richards et al. 1995; Yang and Watt 2005), and although modern human contamination is less of a problem when non-human materials and species-specific sequences are used for DNA research, new materials collected under strict protocols to minimise contamination of any kind are particularly valuable for biochemical research. Many DNA researchers will prefer to do their own sampling of specimens, but in some cases this will not be possible, and at a minimum, the procedure should be supervised by someone familiar with the specimen. Additional knowledge about the storage and treatment history specimens in established collections may be invaluable to researchers, so conservators may play an important role in the sampling process. It is therefore imperative that conservators are aware of the material requirements of samples procured for DNA analysis.

A variety of tissues have been found to be suitable for DNA extraction and analysis. Although bone and teeth (Gilbert et al. 2005c; Thuesen 1995) are often considered provide some extra protection to DNA, other tissues have been successfully used in DNA studies including soft tissue, feather and eggshell (Cann et al. 1993; Cooper 1994), as well as hair (Bonnichsen et al. 2001; Gilbert et al. 2004) and coprolites (Poinar et al. 1998). Every attempt should be made to collect new specimens and samples without contaminating them by using sterile gloves, sterile disposable scalpel blades and tubes (Cooper 1994; Francalacci 1995), particularly when working with human remains. Surgical masks should be worn, and breathing and talking over sample material should be avoided (Cooper 1994; Hagelberg 1994). Ideal samples for DNA analysis are freshly collected, unwashed, uncontaminated teeth or bone (Gilbert et al. 2005c; Hagelberg 1994). Samples intended for DNA analysis should not be washed or in any other way chemically treated, as this may cause contaminating DNA to be carried further into the specimen (Yang and Watt 2005).

Minimise number of people handling specimens and keep a record of everyone who has handled a specimen. When DNA analysis of particular samples is anticipated, DNA samples of anyone handling the samples should also be provided, so that contaminating sequences can be identified (Yang and Watt 2005). This is particularly relevant for freshly collected specimens, as it may be impossible to collect DNA samples from every person who has handled a specimen that has been in a collection for any length of time.

It has been suggested that materials that will be destroyed in the course of sampling should have moulds and casts made as a means of documenting and preserving the morphology of areas

destructively sampled (DeGusta and White 1996). In principle this is a good idea, however, materials applied to the surface of specimens in order to produce moulds and casts may be absorbed into the specimen (Cooper et al. 1994) and may have unknown effects on the sample about to be taken or the future uses of adjacent material. Until the effects of materials on DNA and other biomolecules are better understood, this approach is perhaps best avoided. Additional recording methods, such as photography and possibly laser scanning (DeGusta and White 1996) or photogrammetry, as well as documentation by trained osteologists or zooarchaeologists should be undertaken.

Several studies have assessed various methods to screen bone, in particular, for its suitability in DNA analysis using other preservational characteristics of the bone as a proxy for DNA preservation. Methods suggested include histological characterisation (Colson et al. 1997; Götherström et al. 2002), collagen content (Götherström et al. 2002), amino acid racemization (Poinar 2002), and measuring the extent of modified DNA bases by gas chromatography/mass spectrometry (Höss et al. 1996; Poinar 2002). Although each of these methods can assist in characterising preservation, their use in identifying specimens for sampling cannot guarantee success in DNA analysis. Furthermore, all of these methods require destructive sampling, although some on a scale smaller than for DNA samples. Therefore, their utility may vary depending upon the collection and sampling requirements for the proposed research – if such tests were already undertaken for other research, the results may be useful for sample selection, but if the use of multiple screening tests is suggested prior to DNA sampling the cost and effects of arranging multiple sampling visits may not justify their use in some cases.

As it is often preferable to use subsurface materials for DNA samples to avoid surface contamination by DNA and chemical treatments, different methods may be employed by researchers to remove surface contaminants. Bleaching (Kemp and Smith 2005) and mechanical removal of the surface either by scraping, sandblasting or abrasion are commonplace (Hagelberg 1994). The use of organic solvents to redissolve surface treatments such as consolidants is not recommended, as this may enable treatment chemicals to further penetrate the specimen (Cooper 1994). Furthermore, any decontamination measures should not be undertaken in the field or museum environment, as such methods can only be successful in a controlled environment free from contaminating DNA.

The collection of two samples from different elements or locations on a specimen may be requested for several reasons. Firstly, DNA preservation may vary within a specimen. Secondly, it may be advisable to send separate samples to two separate facilities for DNA extraction and further analysis to ensure reproducibility (Cooper 1994). Thirdly, in cases where specimens may have been in contact with other members of the same or similar species (such as

co-mingled graves or within collection stores), the comparison of sequences obtained from different samples of the same specimen may enable contaminating sequences to be identified (Cooper 1994; Francalacci 1995).

6.4. Review of the screening method developed

The screening test developed for this study is intended to assess the amount of DNA strand breakage due to a particular treatment, enabling treatments to be classified as to their effects on DNA – whether a treatment is damaging, preserving or has a similar effect to water (used as the control). In order to address this question, as many variables as possible must be controlled, to ensure that any effect on DNA preservation can only be attributed to the treatment tested. Therefore, certain restrictions must be imposed on the methods and materials used, which make the experiment somewhat academic, potentially not accurately reflecting the interaction and effects of treatments in real materials. However, it is suggested that this screening method be treated as a starting point to identify treatments that damage DNA *in vitro* in the first instance, and that additional research should follow using other methods of investigation to explore the effects of treatments on DNA in different kinds of tissue. In order to assess the relative merits of this method, as well as acknowledge its drawbacks, a review of the method as developed for this study is warranted.

When devising the method used in this study, it was considered of utmost importance from a conservation standpoint to be able to identify damage directly resulting from chemical treatments as well as to screen several different treatments and for results between treatments to be comparable. It was therefore seen as a requirement to avoid using materials as source DNA that could contain any residues from the burial environment or previous treatments that could interfere with the results of the test. It was also seen as a priority to minimise the amount of sample processing required when performing the test, specifically, DNA extraction and PCR amplification methods were avoided, because damage could occur as a result of extraction methods and amplification could introduce contamination and inhibition issues as well as compensate for damage caused by treatments. Therefore, the use of archaeological tissues or other tissues as a source for DNA were ruled out, as their treatment history would be unknown, additional processing steps and potentially damaging variables would have to be incorporated into the test protocol, the initial concentration of DNA in each sample could not accurately be accounted for making comparisons between treatments somewhat questionable, and subsequently results would not be optimally reproducible. Additionally, a large amount of archaeological material would need to be sacrificed to test several different treatments.

The decision to use PCR products as source DNA eliminated many of the aforementioned problems. Using a master mix of PCR products of known concentration and length as the starting sample of DNA to be treated enabled accurate assessment of the quantity and quality of

target sequences both before and after treatment. In short, DNA damage could be measured and quantified. Experimental error could be minimised (see below) and results were comparable across treatments, as initial samples should be uniform. Reproducibility of the results are also enhanced with this method, as the same PCR products could be generated by other researchers and used in the same way, and the results of other chemicals tested should be comparable with the results of this study. Contamination issues were eliminated by using bare DNA without a diagenetic or treatment history, not using amplification methods in the testing protocol and fluorescently-labelling the PCR products with a dye not used by other researchers in the laboratory. The small volumes of both PCR product samples and treatment solutions required for this method were also beneficial in a number of ways. Small sample volumes of 15 µl of diluted PCR products were readily generated in just a few PCRs, and inclusion of additional fragments of different lengths would be a simple modification. It was possible to screen several replicates of multiple treatments (or multiple concentrations of the same treatment) at the same time under the same environmental conditions, as 96 samples could be contained in a single plate measuring approximately 13 by 8.5 cm. The small volume required of each treatment, only 30 µl, was also a benefit of this method, as both waste and exposure to toxic chemicals were minimised.

A few problems were noted with the method as used in this study, which deserve mention. Evaporation was a problem both with organic solvent-based treatments and heated treatments. Although Parafilm was used to cover the lids of the heated samples, this failed to prevent many of the heated samples evaporating dry by the end of the set exposure period. A different design of PCR plate was used in the repeated heated samples to see if evaporation could be minimised, but this had little effect. Further experimentation with other approaches should be undertaken to minimise evaporation in future studies. This method is also best suited for testing liquid phase treatments, and some problems were encountered with particularly viscous treatment solutions, such as gum arabic, which could not accurately be measured using a Gilson pipette. Viscous treatments should therefore be diluted, and possibly the exposure time lengthened, to address this problem. Problems with solubility were also encountered with some treatments co-precipitating with the DNA, and use of ultrafiltration devices are recommended to get around this problem, as they were found to be helpful in some cases (see Section 4.1.3). Sources of experimental error were primarily reduced to variation in loading solutions by Gilson pipette, as well as some droplet loss prior to the addition of the standard stock that may have occurred with repeated opening of lids on the PCR plate to load interior wells. This was minimised as much as possible by using the same Gilson pipette to load all solutions throughout the experiment to eliminate any variance due to pipette calibration, and by spinning down samples when droplets were present around the lids. These sources of error would be present in any other method devised to assess the effects of treatments on DNA, and are thought to have had minimal effect

on the results. Although a mixer was used to improve contact between the organic solvent-based treatment phase and the aqueous DNA phase, samples were never homogenised. This was not considered to have dramatically affected the results, because adequate contact was achieved to determine that some organic solvent treatments such as mineral oil and turpentine were found to be considerably damaging, but in future mixing of the two phases could possibly be increased by suspending the DNA in a low concentration of ethanol if desired. Lastly, it was found difficult to strictly adhere to the treatment exposure times, particularly very short exposure times such as 25 seconds. Just as all test stock samples were set up at the same time, the standard stock was added to all samples at the same time. However, the amount of time required to ether extract 144 samples was underestimated, and although the samples were refrigerated to slow any ongoing reaction, the treatment exposure time was extended beyond expectation (approximately 8 hours). In future, it is recommended that the standard stock is added to samples at the end of treatment time for aqueous samples and immediately following ether extraction for organic solvent-based samples.

The method developed for this experiment is only intended as a tool to identify treatments that damage DNA, however it is acknowledged that because the effects of treatments on DNA *in vitro* are not necessarily directly comparable to the effects of treatments on DNA in various hard and soft tissues, and that multiple treatments, re-treatment and aged treatments may present additional problems associated with DNA extraction and analysis. Further experiments were intended at the outset of this project, but due to time restrictions could not be pursued. An outline of potential future research intended to more fully examine the effects of preparation and conservation treatments on DNA can be found in section 6.5.

6.5. Suggestions for future research

Although this research has provided some insight into the effects of some chemicals on DNA *in vitro*, further research is required to understand better the complexities associated with DNA preservation in hard and soft collection materials. The effects of treatments on DNA may be buffered by the structure and composition of bone, hair or other tissues, resulting in DNA reacting to treatment in ways not predictable based on the results of this study. Although the cumulative effects of multiple or repeated treatments can be inferred, this was not directly tested and could be of great importance in long-established collections. Due to time restraints, this research also could not explore the effects of treatments in the long-term. Aged treatments may not be readily removed from tissues, may produce unanticipated products upon deterioration and/or may cross-link over time, any of which may affect the ability to extract and analyse DNA. Building on the results of this research, it would also be useful to assess the effects of residual chemical treatment on the extraction or PCR amplification processes "real" samples would undergo – it may be impossible to remove chemicals from samples, which may inhibit amplification of DNA down the line. Understanding which treatments cause damage to DNA

and which do not would enable future studies regarding the identification and minimisation of the PCR inhibiting effects of chemicals. Additional research into sequence specific damage caused by treatments is also important. Each of these topics is worthy of individual research.

If this project were to be repeated, it would be useful to use a portion of each treated sample as template DNA in a PCR to explore the issue of PCR inhibition induced by chemical treatments. This the damaging effects of treatments on DNA as well as the effects of chemical residues on PCR could be ascertained. It was not possible to do this in the course of this study, but is recommended in future.

It is appreciated that the results of the *in vitro* test are in many ways not necessarily a reflection of how DNA might be affected by treatments carried out on tissues in a dry state, for example consolidants drying within and on the surface of bone. Another set of experiments was also originally planned, but due to insufficient time was not pursued, where surface collected animal bone was homogenised and standardised samples of bone powder treated with the same list of treatments tested in this study. Surface collected animal bone was chosen as a source material for DNA because it is relatively common, and any surviving DNA should be at least somewhat fragmented, imitating the condition of DNA in archaeological materials but avoiding the issues surrounding relatively large-scale destructive sampling of archaeological remains. Furthermore, animal bone is subject to fewer ethical restrictions than human bone and also minimises risk of contamination if a species is used that has not previously been sampled in the laboratory where the work is to be carried out. Although treatments would be expected to penetrate homogenised bone powder in a manner different to intact bone, by homogenising the bone, DNA content and condition should be more uniform across samples compared to using bone sections (which could also be done as a separate experiment). Several replicate samples would be set up for each treatment, and samples could be treated (single treatments, repeated treatments and multiple treatments) as well as stored in different microclimates for variable lengths of time, after which DNA from each sample would be extracted and used for PCR amplification. Several target sequences would be selected for PCR amplification varying in length and GC bond content. Such a study could investigate whether the presence of treatment residues affects (or inhibits) the ability to extract and PCR amplify or otherwise analyse DNA. Different extraction and precipitation methods could be tested to optimise the removal of any chemical residues inhibiting analysis. Special attention should be given to treatments known to cross-link as they age, to see if there is any evidence of treatment residues and DNA cross-linking. It would also be possible to begin to examine the buffering effect bone matrix may have on the effects of treatments on DNA when compared to the *in vitro* results of the same treatments. This study would also examine the effects of aged, repeated and multiple treatments, as well as the effects of differences in storage temperature and relative humidity in the storage

environment. Lastly, it would be possible to expand our understanding of sequence specific damage due to chemical treatment.

Chapter 7. Conclusion

In order to assess the effects of preparation and conservation treatments on short strands of DNA so as to determine what treatments may be least damaging to DNA, and therefore preferred for continued use, as well as to assist in predicting the viability of collection material for DNA analysis, a four part research project was undertaken. Firstly, the existing literature related to the effects of chemicals on DNA was reviewed to determine the utility of the methods already employed to investigate the subject. Secondly, a range of preparation and conservation treatments used on both human and animal hard and soft tissues were surveyed to identify chemicals used in the past and their methods of application to collection materials both in the field and in the museum. Thirdly, a screening test was devised to assess the effects of many of the most commonly documented preparation and conservation treatments on short strands of DNA *in vitro* and to quantify the degree of strand breakage sustained by treatment. Lastly, the effects of ancient Egyptian mummification techniques, one of the earliest preparation and conservation treatments, were investigated briefly.

It was discovered that a wide range of treatments have been applied to collection materials in the past. Approximately 475 different chemicals were often used for a variety of purposes, including, but not limited to, the following treatment types: acid preparation, adhesive, adhesive for spirit collections, barrier coat, bleaching agent, chelating agent, cleaning agent, consolidant, degreasing agent, dry soft tissue preservative, drying agent, finishing material, fungicide, moulding/casting material, packing material, pesticide, photographic aid, sealant, skeleton preparation, solvent, and wet soft tissue preservative.

Previous studies aimed at assessing the effects of treatments on DNA used specimens as samples, and the methods employed were best suited to assessing high molecular weight DNA preservation. As archaeological or other material housed in collections over time would tend not to retain high molecular weight DNA, but would instead yield short strands of damaged DNA, existing research methods were not optimally useful, so a new method was developed.

To assess the effects of preparation and conservation treatments on short strands of DNA *in vitro*, a screening test was developed to compare the degree of damage caused by many of the most commonly documented treatments used in the past. Forty-four treatments were selected for screening. Treatments were ranked by the overall percentage of DNA preserved of both sequences combined, and a range of effects can be seen in the treatments tested (see Table 26). "Safe" treatments are defined as those treatments with a mean DNA preservation of 90% or greater and a lower standard deviation limit equal to or greater than 80%, and all other

treatments were deemed "unsafe". The majority of aqueous treatments were found to be more damaging than water alone (the control), as were treatments involving heat. However, a few organic solvents were found to have a minor effect, which was comparable to the control. These results have implications affecting the materials appropriate for use on collection materials for biochemical preservation, as well as predicting the potential preservation of DNA in specimens based upon their treatment history.

Treatment	Overall mean preservation (%)	Overall standard deviation (%)	Standard deviation lower limit (%)
IMS	**100.4**	**8.4**	**92**
Sodium chloride	**100.4**	**11.7**	**88.7**
Trichloroethylene	**100.4**	**8.9**	**91.5**
Water (control)	***100***	***0***	***100***
Water (control) (2)	***100***	***0***	***100***
Toluene	**99.1**	**8.1**	**91**
Amyl acetate	**97.7**	**8.7**	**89**
Acetone	**97.5**	**8.4**	**89.1**
Chloroform	**96.6**	**9**	**87.6**
Xylene	**96.3**	**10**	**86.3**
Arsenic trioxide	**96.2**	**11.5**	**84.7**
1:1 acetone:IMS	**96**	**9.9**	**86.1**
1:1 ethanol:ether	**95.5**	**10.9**	**84.6**
Ethyl acetate	**94.4**	**10.2**	**84.2**
Ethanol	91.7	11.9	79.8
Cellulose nitrate	88.6	12.3	76.3
Kerosene	88.6	9.2	79.4
Methylmethacrylate/ethylacrylate	87.9	6.5	81.4
Carbon tetrachloride	84.4	9.5	74.9
White spirit	82.7	10.5	72.2
Benzene	81.6	8.4	73.2
Ammonium hydroxide	79.6	10.6	69
PVAC/PVAL	75.7	14	61.7
Gasoline	75.3	8.7	66.6
Mercury (II) chloride (2)	42.2	3.8	38.4
Pepsin	27.3	9.1	18.2
Sodium bicarbonate - 24H @ 80°C	25.1	8.7	16.4
Mineral oil	24.8	3.3	21.5
Potassium carbonate - 6H @ 80°C	24.8	10.6	14.2
Pepsin - 48H @ 37°C	21.5	8.4	13.1
Sodium sulfide - 6H @ 80°C	13.9	4.3	9.6
Sodium sulfide	8.9	3.3	5.6
Turpentine	8.6	2.8	5.8
Water (control) - 6H @ 80°C	1.9	0.7	1.2
Other damaging treatments not included above due to a lack of data, or too large standard deviation (greater than 12%): Acetic acid; Acrylic emulsion; Alum; Alum (2); Detergent; Detergent - 40H @ 80°C; Detergent - 40H @ 80°C (2); Detergent (2); Detergent re-ppt; EDTA; EDTA (2); EDTA re-ppt; Gum arabic; Gum arabic re-ppt; Hydrogen peroxide; Linseed oil; Mercury (II) chloride; Mercury (II) chloride re-ppt; Oxalic acid; Oxalic acid (2); Oxalic acid re-ppt; Pepsin - 48H @ 37°C (2); Pepsin (2); Poly(vinyl) butyral resin; Potassium carbonate; PVAC ; Shellac; Sodium bicarbonate; Sodium bicarbonate - 24H @ 80°C (2); Sodium bicarbonate (2); Sodium bicarbonate re-ppt; Sodium carbonate; Sodium chloride - 24H @ 37°C; Sodium hydroxide; Sodium hypochlorite; Sodium hypochlorite (2); Sodium hypochlorite re-ppt; Sodium perborate; Sodium perborate - O/N from 100°C to RT; **Sodium perborate - O/N from 100°C to RT (2); Sodium perborate (2); Sodium perborate re-ppt; Water (control) - 24H** @ 80°C (2); Water (control) - 40H @ 80°C; Water (control) - 40H @ 80°C (2); Water (control) - 48H @ 37°C; Water (control) - 48H @ 37°C (2); Water (control) - O/N from 100°C to RT; Water (control) - O/N from 100°C to RT (2)			

Table 26. Table of tested treatments ranked by overall DNA preservation of both sequences tested. Treatments shaded in grey are safe treatments; unshaded treatments are unsafe treatments. Treatments without sufficient data to calculate the DNA preservation percentage, or with high standard deviations (greater than 12%) are placed at the bottom of the table. "H" = hours, "O/N" = overnight, "RT" = room temperature.

It was also found that DNA damage by preparation and conservation treatments appears to be at

least somewhat sequence specific, with sequences having a higher GC content also having a greater resistance to treatment-induced damage. This potentially has implications for selection of target sequences for biochemical research. Greater success may be had in DNA analyses aimed at studying sequences with a higher GC content. Furthermore, as the effects of treatments were found to vary for two different sequences in this study, with one sequence adversely affected by 31 of the treatments tested and the other sequence adversely affected by only 17, it is recommended that more than one target sequence is studied for any given specimen, to improve the chances of successful DNA analyses.

Both modern and ancient Egyptian animal mummies were used to briefly explore the effects of mummification on DNA. Hair was used as a source of DNA, as it was thought the higher concentration of mitochondrial DNA in hair may enable the extraction of DNA from the ancient specimens. A basic form of ancient Egyptian mummification, consisting of an alcohol wash and several changes of natron was found to have a minimal effect on the DNA of a five year old rabbit mummy. This was in line with the results of similar treatments administered to the SRY 4064 fragment in the screening test (the mummy target sequence and SRY 4064 fragment had comparable GC contents and were expected to be affected by treatments similarly). A single base change from an A to a G in the cloned sequences falls within the range of acceptable alterations. The failure to obtain amplifiable DNA from the ancient Egyptian mummies is not surprising. In addition to the much longer exposure to the Egyptian climate, their conservation history may contribute to the breakdown of DNA, thereby rendering it unamplifiable, in line with published results from other mummy studies.

The practical implications of the results presented here fall into two main categories: the greater inclusion of biochemical preservation into conservation and the consideration of the effect of treatments on sampling. For the few treatments included in both the screening test and the mummy case study, a correlation between the *in vitro* results and the preservation of DNA in a specimen was seen. However, further research is required to determine if DNA in bone and other tissues is buffered by the effects of treatments, and to what degree damaged DNA can be extracted and used for analyses. Nevertheless, the principles of conservation dictate that treatments are to be selected based on having a minimal effect on the integrity of an object and that treatments should not interfere with future analyses. These principles can only be upheld if we better understand the effects preparation and conservation treatments have on the biochemistry of materials in our care. With a greater understanding of the effects of treatments on biomolecules, sampling strategies can then be incorporated into conservation treatment plans and treatments can be devised with the preservation of biomolecules in mind. Enhanced biochemical conservation methods may even be devised.

Although it is hoped that this research can be used to assist in the selection of potential sample material in collections viable for DNA analysis, the results presented here must be used with caution. The list of safe and unsafe treatments should be used only as a *guide* to identify specimens likely to have less damaged DNA rather than to deem specimens outright as unsuitable for sampling. As already mentioned, further research should be done to identify the effects of treatments on DNA in hard and soft tissues, as well as to assess the effects of these treatments on other biochemical research methods.

One overarching problem that was discovered in the course of this study, which by no means is new, was that the treatment history of specimens in collections is often inadequately documented. As a result, it is difficult to use the results of this study to predict with any certainty the suitability of specimens for DNA analysis, with the exception of those recently acquired. Although this problem cannot be resolved retrospectively, the importance of complete and accurate documentation of all methods and materials used on specimens cannot be stressed enough. Additionally, accession and conservation documentation must be readily accessible and adequately cross-referenced. This may not be a novel concept, but to minimise wastage when sampling our irreplaceable cultural and natural heritage, it is vital and deserves reiterating. The utility of the method developed in this study for guiding collection managers in granting sampling permission and for enabling researchers to select specimens for sampling is only as useful as the paper-trail for a specimen in any given collection.

Although a handful of DNA studies have noted that preparation and conservation treatments may affect DNA analyses either by inhibiting DNA extraction and/or PCR amplification (Hall et al. 1992), introducing contaminating DNA from other animal sources (Cooper 1994; Nicholson et al. 2002), or increasing sequence variability (Binladen et al. 2006), the vast majority of studies utilising collection specimens take little account of the potential effects treatments may have on either DNA preservation or the interpretation of results. Due to the lack of communication between DNA researchers and conservators to date, there is little guidance available for the biochemical preservation of irreplaceable collection materials; greater collaboration is necessary. The role of conservators in the future of biochemical research should not be underestimated. Although little may currently be known about the effects of specific treatments on some analyses, conservators should be involved in actively researching this subject. Conservators are in a unique position to improve the quality of research based on collections materials both by improving the conditions under which collections are cared and by using their knowledge of the materials sciences to assist researchers in sampling and devising research strategies. Knowing that conservation decisions may have an impact on the survival of biomolecules and the utility of collection materials in the future requires the principles of conservation to be revisited to ensure the best care is provided to collections on every level.

Glossary

Absorbent: a particulate material used when preparing skins, furs or feathers to soak up blood, wet preparation materials or residual moisture

Acid preparation: the use of a dilute acid (typically 10-15%) to remove matrix affixed to bone or fossils that could not be removed manually or with other standard cleaning solutions. The acid may be applied by brush, but more commonly the specimen is immersed in an acid bath.

Adhesive: a natural or synthetic resin or polymer solution, either aqueous or organic solvent-based, used to mend broken skeletal elements or to affix skin to mounts, etc. The same polymer base may also be used as a consolidant or barrier coat, however an adhesive will have a higher concentration of polymer to solvent.

Adhesive for spirit collections: similar to an adhesive (above), but with the additional properties of being able to endure sustained immersion in solution.

Barrier coat: a dilute resin or polymer solution (typically 10-20%) used to protect exposed fossil or bone material from etching or dissolution during acid preparation, or to protect mends or other elements from other immersion techniques. Barrier coats are usually removed after completion of the treatment for which they are required. The same polymer base may be used at a similar or lower concentration as a consolidant, or at a higher concentration as an adhesive.

Bleaching agent: a material used to whiten skeletal elements for aesthetic purposes. Bleaching agents may be brushed on, or specimens may be immersed in a bleaching bath. Bleaching agents may be used in conjunction with cleaning agents and/or degreasing agents.

Chelating agent: a material used to reduce iron staining

Cleaning agent: used throughout this research to refer to substances used for chemical cleaning (as opposed to manual cleaning) – the use of solutions to remove adhered soil from skeletal elements, or to rinse off biological residues from skins, furs and feathers. Cleaning is not restricted to initial recovery or collection of materials, and may also be a remedial treatment for specimens within collections.

Condolidant: a dilute natural or synthetic resin or polymer solution, either aqueous or organic solvent-based, used to impart strength or to harden fragile materials, such as degraded bone.

The choice of consolidant will depend upon the properties of the bone, particularly whether wet or dry and the environment in which it will be subsequently stored. Consolidants may be applied by brush, spray or immersion, and their penetration may be enhanced by placing the material in a solvent chamber. The same polymer base may also be used as a barrier coat at a similar concentration, or as an adhesive at a higher concentration of polymer to solvent.

Conservation treatment: treatments undertaken with the intention of preservation, and designed using materials and methods in conformance with the conservation principles of minimal intervention, reversibility (whenever possible), preservation of maximum information, maintenance of the state of preservation when recovered or acquisitioned, full documentation and disclosure of materials and methods used. Such treatments may be administered in the field or within collections at any time, and are generally undertaken by trained conservators. The distinction between conservation and preparation treatments is not always clear, as many specimens, particularly freshly collected materials, will undergo some preparation prior to accessioning into collections.

Degreasing agent: a material used to remove biological fats and grease from bones and skins; may be used in conjunction with cleaning agents and/or bleaching agents

Dry soft tissue preservatives: a variety of substances used to maintain soft tissues such as skins, hides, furs, feathers, etc in a dry state, which are often applied in the field whilst collecting specimens. Many multi-purpose mixtures were used which included materials intended to do any number of the following: disinfect, dehydrate and avoid putrefaction, maintain suppleness, repel insects, relax or rehydrate dried hides, or mask smells. Materials were applied as dry rubs, brushed on as a paste, or administered as a bath.

Drying agent: solvents used to dehydrate damp or wet bone, ivory or fossils. Solvent drying may be carried out at the time of recovery or following desalination or wet cleaning.

Finishing materials: primarily substances applied as surface treatments largely for aesthetic purposes when preparing a specimen for exhibition, including clear or tinted coatings applied to skeletal elements, and a variety of paints and pigments applied to soft tissues to enhance their appearance. Some other miscellaneous materials are also included in this category.

Fixative: materials used to stabilise fresh soft tissue, typically either by denaturation or cross-linking proteins, administered either by immersion of the specimen in fluid or injection of the fluid into the specimen. Other preservative solutions may be required for storing tissues in a wet state. For the purposes of this study, all fixatives and preservative solutions have been

jointly considered "wet soft tissue preservatives".

Fungicide: chemicals used to kill mould. Fungicides were added to consolidant solutions to prevent mould growth and were used directly and indirectly (e.g. vapour delivery) on objects during transport as a precautionary measure and as a remedial treatment in collections.

Moulding/casting materials: substances used to replicate bone or fossils. Materials included in this study were those that came into direct contact with specimens, including those used for creating moulds or direct casts and any release agents or similar materials used to coat specimens prior to application of a moulding material.

Packing material: materials used to support or cushion specimens in storage or during transport.

Pesticide: materials used either to repel or kill pests, primarily insects or rodents. Pesticides may be applied directly to specimens as a spray or wipe or by brush. Pesticides may also be applied indirectly as a fumigant, either within a vapour chamber or as a bulk treatment to the entire collection within a building or store room.

Photographic aid: materials applied to whiten, saturate or otherwise enhance the appearance of bones for photography

Polymerase Chain Reaction (PCR): a method used to duplicate DNA for biochemical analysis using samples containing small amounts of DNA by mixing samples of extracted DNA, primers (synthesised DNA sequences used to initiate replication of desired fragments), thermostable polymerase and a solution of nucleotides in a three hour reaction of repetitive denaturing and re-annealing cycles to exponentially duplicate a target sequence of DNA

Preparation treatments: treatments undertaken to render material suitable for exhibition or admission to a collection, typically of freshly collected biological or palaeontological material. Preparation treatments are often undertaken by collectors, amateurs or were passed down to preparators within individual institutions. Preparation treatments may differ from conservation treatments in the materials used (often quite harsh or proprietary), the quantity of materials used (often excessive), or their purpose (often aesthetic).

Preservative solution: a solution used to store wet soft tissue preparations after fixation. For the purposes of this study, all fixatives and preservative solutions have been jointly considered "wet soft tissue preservatives".

Skeleton preparation: refers to a wide range of materials and processes used in preparing fresh or preserved skeletal elements for collections, including hot and cold water maceration, enzyme maceration, chemical maceration, use of dermestid beetles and both pre- and post- dermestid treatments (e.g. baiting materials to entice dermestids and chemical washes to kill any remaining larvae or beetles after processing, respectively), as well as other 'cleaning' treatments to remove biological residues. Treatments expressly for bleaching or degreasing are considered separately, but typically followed skeleton preparations.

Solvent: organic materials used only as a transport medium for other substances, serving no active role in the treatment.

Wet soft tissue preservative: solutions used to maintain soft tissues such as skins, hides, furs, feathers, etc in a wet state. Fixatives may be used in the first instance to initially stabilise soft tissues, and another preservative solution to maintain the specimen in the long-term may also be used. Multiple solutions may be used on the same specimen, and solutions may need changing over time. Different fixatives and preservative solutions affect the properties of soft tissues in different ways, such as the preservation of histological features and color.

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Appendix A: Conservation and preparation literature surveyed

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Appendix B: Mann-Whitney U statistics

Mann-Whitney U statistics. Significance values are highlighted as follows: $.05 \leq p < .01$, $.01 \leq p < .001$, and $.001 \leq p < .000$.

"Unsafe" = the Mann-Whitney U value was small, and $p \leq .05$ for any of the statistics calculated, and for the significant statistics, the median for the control (or unheated treatment, or solvent only, etc) was larger than the median for the treatment (or heated treatment or solvent only, etc).

"Safe" = the Mann-Whitney U value was large, and $p \leq .05$ for none of the statistics calculated OR the Mann-Whitney U value was small, and $p \leq .05$ for any of the statistics calculated, and for the significant statistics, the median for the treatment (or heated treatment or solvent only, etc) was larger than the median for the control (or unheated treatment, or solvent only, etc).

Peak	Treatment	N	Median	Test statistics		Conclusion
SMA1	Water (control)	14	0.934	Mann-Whitney U	0	
	Water (control) - O/N from 100°C to RT	2	0.200	Asymp. Sig. (2-tailed)	.026	
	Total	16		Exact Sig. (2-tailed)	.017	
SMA2	Water (control)	14	0.897	Mann-Whitney U	0	Heating to 100°C and allowing to cool to room temperature overnight = unsafe
	Water (control) - O/N from 100°C to RT	2	0.279	Asymp. Sig. (2-tailed)	.026	
	Total	16		Exact Sig. (2-tailed)	.017	
SMB1	Water (control)	14	0.393	Mann-Whitney U	0	
	Water (control) - O/N from 100°C to RT	2	0.050	Asymp. Sig. (2-tailed)	.026	
	Total	16		Exact Sig. (2-tailed)	.017	
SMB2	Water (control)	14	0.384	Mann-Whitney U	0	
	Water (control) - O/N from 100°C to RT	2	0.146	Asymp. Sig. (2-tailed)	.026	
	Total	16		Exact Sig. (2-tailed)	.017	
TYA1	Water (control)	14	1.818	Mann-Whitney U	0	Heating to 100°C and allowing to cool to room temperature overnight = unsafe
	Water (control) - O/N from 100°C to RT	2	0.954	Asymp. Sig. (2-tailed)	.026	
	Total	16		Exact Sig. (2-tailed)	.017	
TYA2	Water (control)	14	1.848	Mann-Whitney U	0	
	Water (control) - O/N from 100°C to RT	2	1.111	Asymp. Sig. (2-tailed)	.026	
	Total	16		Exact Sig. (2-tailed)	.017	
TYB1	Water (control)	14	2.652	Mann-Whitney U	0	
	Water (control) - O/N from 100°C to RT	2	1.373	Asymp. Sig. (2-tailed)	.026	
	Total	16		Exact Sig. (2-tailed)	.017	
TYB2	Water (control)	14	2.669	Mann-Whitney U	0	
	Water (control) - O/N from 100°C to RT	2	1.592	Asymp. Sig. (2-tailed)	.026	
	Total	16		Exact Sig. (2-tailed)	.017	
TYC1	Water (control)	14	2.108	Mann-Whitney U	0	
	Water (control) - O/N from 100°C to RT	2	1.250	Asymp. Sig. (2-tailed)	.026	
	Total	16		Exact Sig. (2-tailed)	.017	
TYC2	Water (control)	14	2.136	Mann-Whitney U	0	
	Water (control) - O/N from 100°C to RT	2	0.775	Asymp. Sig. (2-tailed)	.026	
	Total	16		Exact Sig. (2-tailed)	.017	
TYD1	Water (control)	14	1.809	Mann-Whitney U	0	
	Water (control) - O/N from 100°C to RT	2	1.018	Asymp. Sig. (2-tailed)	.026	
	Total	16		Exact Sig. (2-tailed)	.017	
TYD2	Water (control)	14	1.832	Mann-Whitney U	0	
	Water (control) - O/N from 100°C to RT	2	0.679	Asymp. Sig. (2-tailed)	.026	
	Total	16		Exact Sig. (2-tailed)	.017	
TYE1	Water (control)	14	4.148	Mann-Whitney U	0	
	Water (control) - O/N from 100°C to RT	2	1.960	Asymp. Sig. (2-tailed)	.026	
	Total	16		Exact Sig. (2-tailed)	.017	

Peak	Treatment	N	Median	Test statistics		Conclusion
TYE2	Water (control)	14	4.094	Mann-Whitney U	0	
	Water (control) - O/N from 100°C to RT	2	1.028	Asymp. Sig. (2-tailed)	.026	
	Total	16		Exact Sig. (2-tailed)	.017	
SMA1	Water (control)	14	0.934	Mann-Whitney U	21.5	1:1 ethanol:ether = safe
	1:1 ethanol:ether	4	1.020	Asymp. Sig. (2-tailed)	.490	
	Total	18		Exact Sig. (2-tailed)	.520	
SMA2	Water (control)	14	0.897	Mann-Whitney U	18	
	1:1 ethanol:ether	4	1.008	Asymp. Sig. (2-tailed)	.288	
	Total	18		Exact Sig. (2-tailed)	.327	
SMB1	Water (control)	14	0.393	Mann-Whitney U	22	
	1:1 ethanol:ether	4	0.444	Asymp. Sig. (2-tailed)	.524	
	Total	18		Exact Sig. (2-tailed)	.574	
SMB2	Water (control)	14	0.384	Mann-Whitney U	19.5	
	1:1 ethanol:ether	4	0.441	Asymp. Sig. (2-tailed)	.366	
	Total	18		Exact Sig. (2-tailed)	.392	
TYA1	Water (control)	14	1.818	Mann-Whitney U	16	1:1 ethanol:ether = safe
	1:1 ethanol:ether	4	1.701	Asymp. Sig. (2-tailed)	.203	
	Total	18		Exact Sig. (2-tailed)	.233	
TYA2	Water (control)	14	1.848	Mann-Whitney U	19	
	1:1 ethanol:ether	4	1.766	Asymp. Sig. (2-tailed)	.339	
	Total	18		Exact Sig. (2-tailed)	.382	
TYB1	Water (control)	14	2.652	Mann-Whitney U	19	
	1:1 ethanol:ether	4	2.481	Asymp. Sig. (2-tailed)	.339	
	Total	18		Exact Sig. (2-tailed)	.382	
TYB2	Water (control)	14	2.669	Mann-Whitney U	16	
	1:1 ethanol:ether	4	2.518	Asymp. Sig. (2-tailed)	.203	
	Total	18		Exact Sig. (2-tailed)	.233	
TYC1	Water (control)	14	2.108	Mann-Whitney U	27	
	1:1 ethanol:ether	4	2.154	Asymp. Sig. (2-tailed)	.915	
	Total	18		Exact Sig. (2-tailed)	.959	
TYC2	Water (control)	14	2.136	Mann-Whitney U	23	
	1:1 ethanol:ether	4	2.184	Asymp. Sig. (2-tailed)	.595	
	Total	18		Exact Sig. (2-tailed)	.645	
TYD1	Water (control)	14	1.809	Mann-Whitney U	19	
	1:1 ethanol:ether	4	1.849	Asymp. Sig. (2-tailed)	.339	
	Total	18		Exact Sig. (2-tailed)	.382	
TYD2	Water (control)	14	1.832	Mann-Whitney U	23.5	
	1:1 ethanol:ether	4	1.885	Asymp. Sig. (2-tailed)	.633	
	Total	18		Exact Sig. (2-tailed)	.659	
TYE1	Water (control)	14	4.148	Mann-Whitney U	23	
	1:1 ethanol:ether	4	4.023	Asymp. Sig. (2-tailed)	.595	
	Total	18		Exact Sig. (2-tailed)	.645	
TYE2	Water (control)	14	4.094	Mann-Whitney U	22	
	1:1 ethanol:ether	4	4.261	Asymp. Sig. (2-tailed)	.524	
	Total	18		Exact Sig. (2-tailed)	.574	
SMA1	Water (control)	14	0.934	Mann-Whitney U	13	1:1 acetone:IMS = safe
	1:1 acetone:IMS	4	0.984	Asymp. Sig. (2-tailed)	.111	
	Total	18		Exact Sig. (2-tailed)	.122	
SMA2	Water (control)	14	0.897	Mann-Whitney U	10	
	1:1 acetone:IMS	4	1.005	Asymp. Sig. (2-tailed)	.056	
	Total	18		Exact Sig. (2-tailed)	.061	
SMB1	Water (control)	14	0.393	Mann-Whitney U	1.5	
	1:1 acetone:IMS	4	0.423	Asymp. Sig. (2-tailed)	.063	
	Total	18		Exact Sig. (2-tailed)	.064	
SMB2	Water (control)	14	0.384	Mann-Whitney U	12.5	
	1:1 acetone:IMS	4	0.434	Asymp. Sig. (2-tailed)	.100	
	Total	18		Exact Sig. (2-tailed)	.105	
TYA1	Water (control)	14	1.818	Mann-Whitney U	25.5	1:1 acetone:IMS = safe
	1:1 acetone:IMS	4	1.713	Asymp. Sig. (2-tailed)	.791	
	Total	18		Exact Sig. (2-tailed)	.813	
TYA2	Water (control)	14	1.848	Mann-Whitney U	16	
	1:1 acetone:IMS	4	1.725	Asymp. Sig. (2-tailed)	.203	

Peak	Treatment	N	Median	Test statistics		Conclusion
	Total	18		Exact Sig. (2-tailed)	.233	
TYB1	Water (control)	14	2.652	Mann-Whitney U	18	
	1:1 acetone:IMS	4	2.406	Asymp. Sig. (2-tailed)	.288	
	Total	18		Exact Sig. (2-tailed)	.327	
TYB2	Water (control)	14	2.669	Mann-Whitney U	17	
	1:1 acetone:IMS	4	2.469	Asymp. Sig. (2-tailed)	.243	
	Total	18		Exact Sig. (2-tailed)	.277	
TYC1	Water (control)	14	2.108	Mann-Whitney U	28	
	1:1 acetone:IMS	4	2.067	Asymp. Sig. (2-tailed)	1.000	
	Total	18		Exact Sig. (2-tailed)	1.000	
TYC2	Water (control)	14	2.136	Mann-Whitney U	21	
	1:1 acetone:IMS	4	2.168	Asymp. Sig. (2-tailed)	.457	
	Total	18		Exact Sig. (2-tailed)	.505	
TYD1	Water (control)	14	1.809	Mann-Whitney U	18	
	1:1 acetone:IMS	4	1.926	Asymp. Sig. (2-tailed)	.288	
	Total	18		Exact Sig. (2-tailed)	.327	
TYD2	Water (control)	14	1.832	Mann-Whitney U	22	
	1:1 acetone:IMS	4	1.879	Asymp. Sig. (2-tailed)	.524	
	Total	18		Exact Sig. (2-tailed)	.574	
TYE1	Water (control)	14	4.148	Mann-Whitney U	17	
	1:1 acetone:IMS	4	3.678	Asymp. Sig. (2-tailed)	.243	
	Total	18		Exact Sig. (2-tailed)	.277	
TYE2	Water (control)	14	4.094	Mann-Whitney U	12	
	1:1 acetone:IMS	4	3.778	Asymp. Sig. (2-tailed)	.089	
	Total	18		Exact Sig. (2-tailed)	.101	
SMA1	Water (control)	14	0.934	Mann-Whitney U	0	
	Acetic acid	4	0.196	Asymp. Sig. (2-tailed)	.003	
	Total	18		Exact Sig. (2-tailed)	.001	
SMA2	Water (control)	14	0.897	Mann-Whitney U	0	
	Acetic acid	4	0.190	Asymp. Sig. (2-tailed)	.003	
	Total	18		Exact Sig. (2-tailed)	.001	Acetic acid = unsafe
SMB1	Water (control)	14	0.393	Mann-Whitney U	0	
	Acetic acid	4	0.038	Asymp. Sig. (2-tailed)	.003	
	Total	18		Exact Sig. (2-tailed)	.001	
SMB2	Water (control)	14	0.384	Mann-Whitney U	0	
	Acetic acid	4	0.038	Asymp. Sig. (2-tailed)	.003	
	Total	18		Exact Sig. (2-tailed)	.001	
TYA1	Water (control)	14	1.818	Mann-Whitney U	0	Acetic acid = unsafe
	Acetic acid	4	0.870	Asymp. Sig. (2-tailed)	.003	
	Total	18		Exact Sig. (2-tailed)	.001	
TYA2	Water (control)	14	1.848	Mann-Whitney U	0	
	Acetic acid	4	0.878	Asymp. Sig. (2-tailed)	.003	
	Total	18		Exact Sig. (2-tailed)	.001	
TYB1	Water (control)	14	2.652	Mann-Whitney U	0	
	Acetic acid	4	1.296	Asymp. Sig. (2-tailed)	.003	
	Total	18		Exact Sig. (2-tailed)	.001	
TYB2	Water (control)	14	2.669	Mann-Whitney U	0	
	Acetic acid	4	1.298	Asymp. Sig. (2-tailed)	.003	
	Total	18		Exact Sig. (2-tailed)	.001	
TYC1	Water (control)	14	2.108	Mann-Whitney U	0	
	Acetic acid	4	1.031	Asymp. Sig. (2-tailed)	.003	
	Total	18		Exact Sig. (2-tailed)	.001	
TYC2	Water (control)	14	2.136	Mann-Whitney U	0	
	Acetic acid	4	1.103	Asymp. Sig. (2-tailed)	.003	
	Total	18		Exact Sig. (2-tailed)	.001	
TYD1	Water (control)	14	1.809	Mann-Whitney U	0	
	Acetic acid	4	0.935	Asymp. Sig. (2-tailed)	.003	
	Total	18		Exact Sig. (2-tailed)	.001	
TYD2	Water (control)	14	1.832	Mann-Whitney U	0	
	Acetic acid	4	1.035	Asymp. Sig. (2-tailed)	.003	
	Total	18		Exact Sig. (2-tailed)	.001	
TYE1	Water (control)	14	4.148	Mann-Whitney U	2	

Peak	Treatment	N	Median	Test statistics		Conclusion
	Acetic acid	4	2.473	Asymp. Sig. (2-tailed)	.006	
	Total	18		Exact Sig. (2-tailed)	.003	
	Water (control)	14	4.094	Mann-Whitney U	3	
TYE2	Acetic acid	4	2.856	Asymp. Sig. (2-tailed)	.008	
	Total	18		Exact Sig. (2-tailed)	.005	
	Water (control)	14	0.934	Mann-Whitney U	26	
SMA1	Acetone	4	0.952	Asymp. Sig. (2-tailed)	.832	
	Total	18		Exact Sig. (2-tailed)	.863	
	Water (control)	14	0.897	Mann-Whitney U	18	
SMA2	Acetone	4	0.914	Asymp. Sig. (2-tailed)	.288	
	Total	18		Exact Sig. (2-tailed)	.318	
	Water (control)	14	0.393	Mann-Whitney U	1.5	Acetone = safe
SMB1	Acetone	4	0.433	Asymp. Sig. (2-tailed)	.063	
	Total	18		Exact Sig. (2-tailed)	.064	
	Water (control)	14	0.384	Mann-Whitney U	13	
SMB2	Acetone	4	0.425	Asymp. Sig. (2-tailed)	.111	
	Total	18		Exact Sig. (2-tailed)	.127	
	Water (control)	14	1.818	Mann-Whitney U	24	
TYA1	Acetone	4	1.835	Asymp. Sig. (2-tailed)	.671	
	Total	18		Exact Sig. (2-tailed)	.721	
	Water (control)	14	1.848	Mann-Whitney U	28	
TYA2	Acetone	4	1.839	Asymp. Sig. (2-tailed)	1.000	
	Total	18		Exact Sig. (2-tailed)	1.000	
	Water (control)	14	2.652	Mann-Whitney U	26	
TYB1	Acetone	4	2.616	Asymp. Sig. (2-tailed)	.832	
	Total	18		Exact Sig. (2-tailed)	.878	
	Water (control)	14	2.669	Mann-Whitney U	27	
TYB2	Acetone	4	2.619	Asymp. Sig. (2-tailed)	.915	
	Total	18		Exact Sig. (2-tailed)	.959	
	Water (control)	14	2.108	Mann-Whitney U	24	
TYC1	Acetone	4	2.229	Asymp. Sig. (2-tailed)	.671	
	Total	18		Exact Sig. (2-tailed)	.721	Acetone = safe
	Water (control)	14	2.136	Mann-Whitney U	22	
TYC2	Acetone	4	2.264	Asymp. Sig. (2-tailed)	.524	
	Total	18		Exact Sig. (2-tailed)	.574	
	Water (control)	14	1.809	Mann-Whitney U	16	
TYD1	Acetone	4	1.985	Asymp. Sig. (2-tailed)	.203	
	Total	18		Exact Sig. (2-tailed)	.233	
	Water (control)	14	1.832	Mann-Whitney U	21.5	
TYD2	Acetone	4	1.918	Asymp. Sig. (2-tailed)	.490	
	Total	18		Exact Sig. (2-tailed)	.518	
	Water (control)	14	4.148	Mann-Whitney U	24	
TYE1	Acetone	4	3.937	Asymp. Sig. (2-tailed)	.671	
	Total	18		Exact Sig. (2-tailed)	.721	
	Water (control)	14	4.094	Mann-Whitney U	22	
TYE2	Acetone	4	3.999	Asymp. Sig. (2-tailed)	.524	
	Total	18		Exact Sig. (2-tailed)	.574	
	Water (control)	14	0.934	Mann-Whitney U	3	
SMA1	Acrylic emulsion	3	1.170	Asymp. Sig. (2-tailed)	.023	
	Total	17		Exact Sig. (2-tailed)	.019	
	Water (control)	14	0.897	Mann-Whitney U	3	
SMA2	Acrylic emulsion	4	1.239	Asymp. Sig. (2-tailed)	.008	
	Total	18		Exact Sig. (2-tailed)	.005	Acrylic
	Water (control)	14	0.393	Mann-Whitney U	0	emulsion = safe
SMB1	Acrylic emulsion	3	0.661	Asymp. Sig. (2-tailed)	.008	
	Total	17		Exact Sig. (2-tailed)	.003	
	Water (control)	14	0.384	Mann-Whitney U	0	
SMB2	Acrylic emulsion	4	0.680	Asymp. Sig. (2-tailed)	.003	
	Total	18		Exact Sig. (2-tailed)	.001	
	Water (control)	14	1.818	Mann-Whitney U	18	Acrylic
TYA1	Acrylic emulsion	3	1.808	Asymp. Sig. (2-tailed)	.705	emulsion =
	Total	17		Exact Sig. (2-tailed)	.768	unsafe

Peak	Treatment	N	Median	Test statistics		Conclusion
TYA2	Water (control)	14	1.848	Mann-Whitney U	27	
	Acrylic emulsion	4	1.877	Asymp. Sig. (2-tailed)	.915	
	Total	18		Exact Sig. (2-tailed)	.959	
TYB1	Water (control)	14	2.652	Mann-Whitney U	15	
	Acrylic emulsion	3	2.541	Asymp. Sig. (2-tailed)	.450	
	Total	17		Exact Sig. (2-tailed)	.509	
TYB2	Water (control)	14	2.669	Mann-Whitney U	11	
	Acrylic emulsion	4	2.450	Asymp. Sig. (2-tailed)	.071	
	Total	18		Exact Sig. (2-tailed)	.079	
TYC1	Water (control)	14	2.108	Mann-Whitney U	6	
	Acrylic emulsion	3	1.205	Asymp. Sig. (2-tailed)	.059	
	Total	17		Exact Sig. (2-tailed)	.068	
TYC2	Water (control)	14	2.136	Mann-Whitney U	0	
	Acrylic emulsion	4	1.225	Asymp. Sig. (2-tailed)	.003	
	Total	18		Exact Sig. (2-tailed)	.001	
TYD1	Water (control)	14	1.809	Mann-Whitney U	14	
	Acrylic emulsion	3	1.270	Asymp. Sig. (2-tailed)	.378	
	Total	17		Exact Sig. (2-tailed)	.432	
TYD2	Water (control)	14	1.832	Mann-Whitney U	0	
	Acrylic emulsion	4	1.322	Asymp. Sig. (2-tailed)	.003	
	Total	18		Exact Sig. (2-tailed)	.001	
TYE1	Water (control)	14	4.148	Mann-Whitney U	2	
	Acrylic emulsion	3	1.880	Asymp. Sig. (2-tailed)	.017	
	Total	17		Exact Sig. (2-tailed)	.012	
TYE2	Water (control)	14	4.094	Mann-Whitney U	1	
	Acrylic emulsion	4	1.797	Asymp. Sig. (2-tailed)	.004	
	Total	18		Exact Sig. (2-tailed)	.001	
SMA1	Water (control)	14	0.934	Mann-Whitney U	14	
	Ammonium hydroxide	4	0.812	Asymp. Sig. (2-tailed)	.137	
	Total	18		Exact Sig. (2-tailed)	.151	
SMA2	Water (control)	14	0.897	Mann-Whitney U	19	
	Ammonium hydroxide	4	0.771	Asymp. Sig. (2-tailed)	.339	
	Total	18		Exact Sig. (2-tailed)	.382	Ammonium hydroxide = safe
SMB1	Water (control)	14	0.393	Mann-Whitney U	25	
	Ammonium hydroxide	4	0.403	Asymp. Sig. (2-tailed)	.750	
	Total	18		Exact Sig. (2-tailed)	.798	
SMB2	Water (control)	14	0.384	Mann-Whitney U	20	
	Ammonium hydroxide	4	0.315	Asymp. Sig. (2-tailed)	.396	
	Total	18		Exact Sig. (2-tailed)	.442	
TYA1	Water (control)	14	1.818	Mann-Whitney U	6	Ammonium hydroxide = unsafe
	Ammonium hydroxide	4	1.496	Asymp. Sig. (2-tailed)	.019	
	Total	18		Exact Sig. (2-tailed)	.018	
TYA2	Water (control)	14	1.848	Mann-Whitney U	0	
	Ammonium hydroxide	4	1.438	Asymp. Sig. (2-tailed)	.003	
	Total	18		Exact Sig. (2-tailed)	.001	
TYB1	Water (control)	14	2.652	Mann-Whitney U	3	
	Ammonium hydroxide	4	2.175	Asymp. Sig. (2-tailed)	.008	
	Total	18		Exact Sig. (2-tailed)	.005	
TYB2	Water (control)	14	2.669	Mann-Whitney U	1.5	
	Ammonium hydroxide	4	2.139	Asymp. Sig. (2-tailed)	.005	
	Total	18		Exact Sig. (2-tailed)	.002	
TYC1	Water (control)	14	2.108	Mann-Whitney U	15	
	Ammonium hydroxide	4	1.872	Asymp. Sig. (2-tailed)	.167	
	Total	18		Exact Sig. (2-tailed)	.192	
TYC2	Water (control)	14	2.136	Mann-Whitney U	4	
	Ammonium hydroxide	4	1.817	Asymp. Sig. (2-tailed)	.011	
	Total	18		Exact Sig. (2-tailed)	.008	
TYD1	Water (control)	14	1.809	Mann-Whitney U	3	
	Ammonium hydroxide	4	1.551	Asymp. Sig. (2-tailed)	.008	
	Total	18		Exact Sig. (2-tailed)	.005	
TYD2	Water (control)	14	1.832	Mann-Whitney U	0	
	Ammonium hydroxide	4	1.540	Asymp. Sig. (2-tailed)	.003	

Peak	Treatment	N	Median	Test statistics		Conclusion
	Total	18		Exact Sig. (2-tailed)	.001	
TYE1	Water (control)	14	4.148	Mann-Whitney U	8	
	Ammonium hydroxide	4	3.102	Asymp. Sig. (2-tailed)	.034	
	Total	18		Exact Sig. (2-tailed)	.035	
TYE2	Water (control)	14	4.094	Mann-Whitney U	21	
	Ammonium hydroxide	4	3.859	Asymp. Sig. (2-tailed)	.457	
	Total	18		Exact Sig. (2-tailed)	.505	
SMA1	Water (control)	14	0.934	Mann-Whitney U	20	
	Amyl acetate	4	0.902	Asymp. Sig. (2-tailed)	.395	
	Total	18		Exact Sig. (2-tailed)	.430	
SMA2	Water (control)	14	0.897	Mann-Whitney U	16	
	Amyl acetate	4	0.863	Asymp. Sig. (2-tailed)	.203	
	Total	18		Exact Sig. (2-tailed)	.233	Amyl acetate = safe
SMB1	Water (control)	14	0.393	Mann-Whitney U	25	
	Amyl acetate	4	0.416	Asymp. Sig. (2-tailed)	.750	
	Total	18		Exact Sig. (2-tailed)	.798	
SMB2	Water (control)	14	0.384	Mann-Whitney U	23.5	
	Amyl acetate	4	0.399	Asymp. Sig. (2-tailed)	.633	
	Total	18		Exact Sig. (2-tailed)	.658	
TYA1	Water (control)	14	1.818	Mann-Whitney U	22	
	Amyl acetate	4	1.861	Asymp. Sig. (2-tailed)	.524	
	Total	18		Exact Sig. (2-tailed)	.574	
TYA2	Water (control)	14	1.848	Mann-Whitney U	25	
	Amyl acetate	4	1.872	Asymp. Sig. (2-tailed)	.750	
	Total	18		Exact Sig. (2-tailed)	.798	
TYB1	Water (control)	14	2.652	Mann-Whitney U	24	
	Amyl acetate	4	2.677	Asymp. Sig. (2-tailed)	.671	
	Total	18		Exact Sig. (2-tailed)	.721	
TYB2	Water (control)	14	2.669	Mann-Whitney U	27	
	Amyl acetate	4	2.701	Asymp. Sig. (2-tailed)	.915	
	Total	18		Exact Sig. (2-tailed)	.959	
TYC1	Water (control)	14	2.108	Mann-Whitney U	20	
	Amyl acetate	4	2.304	Asymp. Sig. (2-tailed)	.396	
	Total	18		Exact Sig. (2-tailed)	.442	Amyl acetate = safe
TYC2	Water (control)	14	2.136	Mann-Whitney U	20	
	Amyl acetate	4	2.351	Asymp. Sig. (2-tailed)	.396	
	Total	18		Exact Sig. (2-tailed)	.442	
TYD1	Water (control)	14	1.809	Mann-Whitney U	7	
	Amyl acetate	4	2.068	Asymp. Sig. (2-tailed)	.026	
	Total	18		Exact Sig. (2-tailed)	.025	
TYD2	Water (control)	14	1.832	Mann-Whitney U	10	
	Amyl acetate	4	2.061	Asymp. Sig. (2-tailed)	.056	
	Total	18		Exact Sig. (2-tailed)	.061	
TYE1	Water (control)	14	4.148	Mann-Whitney U	26	
	Amyl acetate	4	4.037	Asymp. Sig. (2-tailed)	.832	
	Total	18		Exact Sig. (2-tailed)	.878	
TYE2	Water (control)	14	4.094	Mann-Whitney U	21	
	Amyl acetate	4	4.161	Asymp. Sig. (2-tailed)	.457	
	Total	18		Exact Sig. (2-tailed)	.505	
SMA1	Water (control)	14	0.934	Mann-Whitney U	25	
	Arsenic trioxide	4	0.963	Asymp. Sig. (2-tailed)	.750	
	Total	18		Exact Sig. (2-tailed)	.783	
SMA2	Water (control)	14	0.897	Mann-Whitney U	22	
	Arsenic trioxide	4	0.917	Asymp. Sig. (2-tailed)	.524	
	Total	18		Exact Sig. (2-tailed)	.574	Arsenic trioxide = safe
SMB1	Water (control)	14	0.393	Mann-Whitney U	22	
	Arsenic trioxide	4	0.429	Asymp. Sig. (2-tailed)	.524	
	Total	18		Exact Sig. (2-tailed)	.574	
SMB2	Water (control)	14	0.384	Mann-Whitney U	27	
	Arsenic trioxide	4	0.398	Asymp. Sig. (2-tailed)	.915	
	Total	18		Exact Sig. (2-tailed)	.959	

Peak	Treatment	N	Median	Test statistics		Conclusion
TYA1	Water (control)	14	1.818	Mann-Whitney U	22	
	Arsenic trioxide	4	1.728	Asymp. Sig. (2-tailed)	.524	
	Total	18		Exact Sig. (2-tailed)	.574	
TYA2	Water (control)	14	1.848	Mann-Whitney U	16	
	Arsenic trioxide	4	1.798	Asymp. Sig. (2-tailed)	.203	
	Total	18		Exact Sig. (2-tailed)	.233	
TYB1	Water (control)	14	2.652	Mann-Whitney U	23	
	Arsenic trioxide	4	2.533	Asymp. Sig. (2-tailed)	.595	
	Total	18		Exact Sig. (2-tailed)	.645	
TYB2	Water (control)	14	2.669	Mann-Whitney U	16	
	Arsenic trioxide	4	2.573	Asymp. Sig. (2-tailed)	.203	
	Total	18		Exact Sig. (2-tailed)	.233	
TYC1	Water (control)	14	2.108	Mann-Whitney U	24	
	Arsenic trioxide	4	2.085	Asymp. Sig. (2-tailed)	.671	
	Total	18		Exact Sig. (2-tailed)	.721	Arsenic
TYC2	Water (control)	14	2.136	Mann-Whitney U	28	trioxide = safe
	Arsenic trioxide	4	2.225	Asymp. Sig. (2-tailed)	1.000	
	Total	18		Exact Sig. (2-tailed)	1.000	
TYD1	Water (control)	14	1.809	Mann-Whitney U	18	
	Arsenic trioxide	4	1.972	Asymp. Sig. (2-tailed)	.288	
	Total	18		Exact Sig. (2-tailed)	.327	
TYD2	Water (control)	14	1.832	Mann-Whitney U	12	
	Arsenic trioxide	4	2.040	Asymp. Sig. (2-tailed)	.089	
	Total	18		Exact Sig. (2-tailed)	.101	
TYE1	Water (control)	14	4.148	Mann-Whitney U	18.5	
	Arsenic trioxide	4	4.318	Asymp. Sig. (2-tailed)	.313	
	Total	18		Exact Sig. (2-tailed)	.337	
TYE2	Water (control)	14	4.094	Mann-Whitney U	11	
	Arsenic trioxide	4	4.547	Asymp. Sig. (2-tailed)	.071	
	Total	18		Exact Sig. (2-tailed)	.079	
SMA1	Water (control)	14	0.934	Mann-Whitney U	0	
	Benzene	4	0.729	Asymp. Sig. (2-tailed)	.003	
	Total	18		Exact Sig. (2-tailed)	.001	
SMA2	Water (control)	14	0.897	Mann-Whitney U	2	
	Benzene	4	0.711	Asymp. Sig. (2-tailed)	.006	
	Total	18		Exact Sig. (2-tailed)	.003	Benzene =
SMB1	Water (control)	14	0.393	Mann-Whitney U	0	unsafe
	Benzene	4	0.319	Asymp. Sig. (2-tailed)	.003	
	Total	18		Exact Sig. (2-tailed)	.001	
SMB2	Water (control)	14	0.384	Mann-Whitney U	1	
	Benzene	4	0.298	Asymp. Sig. (2-tailed)	.004	
	Total	18		Exact Sig. (2-tailed)	.001	
TYA1	Water (control)	14	1.818	Mann-Whitney U	8	Benzene =
	Benzene	4	1.548	Asymp. Sig. (2-tailed)	.034	unsafe
	Total	18		Exact Sig. (2-tailed)	.035	
TYA2	Water (control)	14	1.848	Mann-Whitney U	5	
	Benzene	4	1.548	Asymp. Sig. (2-tailed)	.015	
	Total	18		Exact Sig. (2-tailed)	.012	
TYB1	Water (control)	14	2.652	Mann-Whitney U	8	
	Benzene	4	2.229	Asymp. Sig. (2-tailed)	.034	
	Total	18		Exact Sig. (2-tailed)	.035	
TYB2	Water (control)	14	2.669	Mann-Whitney U	5	
	Benzene	4	2.257	Asymp. Sig. (2-tailed)	.015	
	Total	18		Exact Sig. (2-tailed)	.012	
TYC1	Water (control)	14	2.108	Mann-Whitney U	10	
	Benzene	4	1.872	Asymp. Sig. (2-tailed)	.056	
	Total	18		Exact Sig. (2-tailed)	.061	
TYC2	Water (control)	14	2.136	Mann-Whitney U	7	
	Benzene	4	1.985	Asymp. Sig. (2-tailed)	.026	
	Total	18		Exact Sig. (2-tailed)	.025	
TYD1	Water (control)	14	1.809	Mann-Whitney U	19	

Peak	Treatment	N	Median	Test statistics		Conclusion
	Benzene	4	1.707	Asymp. Sig. (2-tailed)	.339	
	Total	18		Exact Sig. (2-tailed)	.382	
TYD2	Water (control)	14	1.832	Mann-Whitney U	19	
	Benzene	4	1.729	Asymp. Sig. (2-tailed)	.339	
	Total	18		Exact Sig. (2-tailed)	.382	
TYE1	Water (control)	14	4.148	Mann-Whitney U	10	
	Benzene	4	3.397	Asymp. Sig. (2-tailed)	.056	
	Total	18		Exact Sig. (2-tailed)	.061	
TYE2	Water (control)	14	4.094	Mann-Whitney U	12	
	Benzene	4	3.507	Asymp. Sig. (2-tailed)	.089	
	Total	18		Exact Sig. (2-tailed)	.101	
SMA1	Water (control)	14	0.934	Mann-Whitney U	1	Carbon tetrachloride = unsafe
	Carbon tetrachloride	4	0.774	Asymp. Sig. (2-tailed)	.004	
	Total	18		Exact Sig. (2-tailed)	.001	
SMA2	Water (control)	14	0.897	Mann-Whitney U	3	
	Carbon tetrachloride	4	0.760	Asymp. Sig. (2-tailed)	.008	
	Total	18		Exact Sig. (2-tailed)	.005	
SMB1	Water (control)	14	0.393	Mann-Whitney U	1	
	Carbon tetrachloride	4	0.344	Asymp. Sig. (2-tailed)	.004	
	Total	18		Exact Sig. (2-tailed)	.001	
SMB2	Water (control)	14	0.384	Mann-Whitney U	3	
	Carbon tetrachloride	4	0.323	Asymp. Sig. (2-tailed)	.008	
	Total	18		Exact Sig. (2-tailed)	.005	
TYA1	Water (control)	14	1.818	Mann-Whitney U	10	Carbon tetrachloride = unsafe
	Carbon tetrachloride	4	1.605	Asymp. Sig. (2-tailed)	.056	
	Total	18		Exact Sig. (2-tailed)	.059	
TYA2	Water (control)	14	1.848	Mann-Whitney U	6	
	Carbon tetrachloride	4	1.620	Asymp. Sig. (2-tailed)	.019	
	Total	18		Exact Sig. (2-tailed)	.018	
TYB1	Water (control)	14	2.652	Mann-Whitney U	6	
	Carbon tetrachloride	4	2.308	Asymp. Sig. (2-tailed)	.019	
	Total	18		Exact Sig. (2-tailed)	.018	
TYB2	Water (control)	14	2.669	Mann-Whitney U	8	
	Carbon tetrachloride	4	2.375	Asymp. Sig. (2-tailed)	.034	
	Total	18		Exact Sig. (2-tailed)	.035	
TYC1	Water (control)	14	2.108	Mann-Whitney U	8	
	Carbon tetrachloride	4	1.875	Asymp. Sig. (2-tailed)	.034	
	Total	18		Exact Sig. (2-tailed)	.035	
TYC2	Water (control)	14	2.136	Mann-Whitney U	8	
	Carbon tetrachloride	4	2.007	Asymp. Sig. (2-tailed)	.034	
	Total	18		Exact Sig. (2-tailed)	.035	
TYD1	Water (control)	14	1.809	Mann-Whitney U	18	
	Carbon tetrachloride	4	1.731	Asymp. Sig. (2-tailed)	.288	
	Total	18		Exact Sig. (2-tailed)	.327	
TYD2	Water (control)	14	1.832	Mann-Whitney U	27	
	Carbon tetrachloride	4	1.814	Asymp. Sig. (2-tailed)	.915	
	Total	18		Exact Sig. (2-tailed)	.959	
TYE1	Water (control)	14	4.148	Mann-Whitney U	11	
	Carbon tetrachloride	4	3.669	Asymp. Sig. (2-tailed)	.071	
	Total	18		Exact Sig. (2-tailed)	.079	
TYE2	Water (control)	14	4.094	Mann-Whitney U	21	
	Carbon tetrachloride	4	3.946	Asymp. Sig. (2-tailed)	.457	
	Total	18		Exact Sig. (2-tailed)	.505	
SMA1	Water (control)	14	0.934	Mann-Whitney U	25	Cellulose nitrate = safe
	Cellulose nitrate	4	0.901	Asymp. Sig. (2-tailed)	.750	
	Total	18		Exact Sig. (2-tailed)	.783	
SMA2	Water (control)	14	0.897	Mann-Whitney U	26	
	Cellulose nitrate	4	0.875	Asymp. Sig. (2-tailed)	.832	
	Total	18		Exact Sig. (2-tailed)	.878	
SMB1	Water (control)	14	0.393	Mann-Whitney U	27	
	Cellulose nitrate	4	0.408	Asymp. Sig. (2-tailed)	.915	
	Total	18		Exact Sig. (2-tailed)	.959	

Peak	Treatment	N	Median	Test statistics		Conclusion
SMB2	Water (control)	14	0.384	Mann-Whitney U	27	
	Cellulose nitrate	4	0.377	Asymp. Sig. (2-tailed)	.915	
	Total	18		Exact Sig. (2-tailed)	.937	
TYA1	Water (control)	14	1.818	Mann-Whitney U	12	
	Cellulose nitrate	4	1.460	Asymp. Sig. (2-tailed)	.089	
	Total	18		Exact Sig. (2-tailed)	.101	
TYA2	Water (control)	14	1.848	Mann-Whitney U	9	
	Cellulose nitrate	4	1.421	Asymp. Sig. (2-tailed)	.044	
	Total	18		Exact Sig. (2-tailed)	.044	
TYB1	Water (control)	14	2.652	Mann-Whitney U	11	
	Cellulose nitrate	4	2.192	Asymp. Sig. (2-tailed)	.071	
	Total	18		Exact Sig. (2-tailed)	.079	
TYB2	Water (control)	14	2.669	Mann-Whitney U	11	
	Cellulose nitrate	4	2.057	Asymp. Sig. (2-tailed)	.071	
	Total	18		Exact Sig. (2-tailed)	.079	
TYC1	Water (control)	14	2.108	Mann-Whitney U	15	
	Cellulose nitrate	4	1.822	Asymp. Sig. (2-tailed)	.167	
	Total	18		Exact Sig. (2-tailed)	.192	Cellulose
TYC2	Water (control)	14	2.136	Mann-Whitney U	9	nitrate = unsafe
	Cellulose nitrate	4	1.711	Asymp. Sig. (2-tailed)	.044	
	Total	18		Exact Sig. (2-tailed)	.046	
TYD1	Water (control)	14	1.809	Mann-Whitney U	26	
	Cellulose nitrate	4	1.780	Asymp. Sig. (2-tailed)	.832	
	Total	18		Exact Sig. (2-tailed)	.878	
TYD2	Water (control)	14	1.832	Mann-Whitney U	15	
	Cellulose nitrate	4	1.674	Asymp. Sig. (2-tailed)	.167	
	Total	18		Exact Sig. (2-tailed)	.192	
TYE1	Water (control)	14	4.148	Mann-Whitney U	18	
	Cellulose nitrate	4	3.641	Asymp. Sig. (2-tailed)	.288	
	Total	18		Exact Sig. (2-tailed)	.327	
TYE2	Water (control)	14	4.094	Mann-Whitney U	12	
	Cellulose nitrate	4	3.484	Asymp. Sig. (2-tailed)	.089	
	Total	18		Exact Sig. (2-tailed)	.101	
SMA1	Water (control)	14	0.934	Mann-Whitney U	24	
	Chloroform	4	0.905	Asymp. Sig. (2-tailed)	.671	
	Total	18		Exact Sig. (2-tailed)	.706	
SMA2	Water (control)	14	0.897	Mann-Whitney U	19	
	Chloroform	4	0.849	Asymp. Sig. (2-tailed)	.339	
	Total	18		Exact Sig. (2-tailed)	.382	Chloroform =
SMB1	Water (control)	14	0.393	Mann-Whitney U	23.5	safe
	Chloroform	4	0.407	Asymp. Sig. (2-tailed)	.633	
	Total	18		Exact Sig. (2-tailed)	.660	
SMB2	Water (control)	14	0.384	Mann-Whitney U	25	
	Chloroform	4	0.400	Asymp. Sig. (2-tailed)	.750	
	Total	18		Exact Sig. (2-tailed)	.798	
TYA1	Water (control)	14	1.818	Mann-Whitney U	23.5	Chloroform =
	Chloroform	4	1.711	Asymp. Sig. (2-tailed)	.633	safe
	Total	18		Exact Sig. (2-tailed)	.659	
TYA2	Water (control)	14	1.848	Mann-Whitney U	22	
	Chloroform	4	1.780	Asymp. Sig. (2-tailed)	.524	
	Total	18		Exact Sig. (2-tailed)	.574	
TYB1	Water (control)	14	2.652	Mann-Whitney U	22	
	Chloroform	4	2.468	Asymp. Sig. (2-tailed)	.524	
	Total	18		Exact Sig. (2-tailed)	.574	
TYB2	Water (control)	14	2.669	Mann-Whitney U	22	
	Chloroform	4	2.574	Asymp. Sig. (2-tailed)	.524	
	Total	18		Exact Sig. (2-tailed)	.574	
TYC1	Water (control)	14	2.108	Mann-Whitney U	26	
	Chloroform	4	2.060	Asymp. Sig. (2-tailed)	.832	
	Total	18		Exact Sig. (2-tailed)	.878	
TYC2	Water (control)	14	2.136	Mann-Whitney U	25	
	Chloroform	4	2.307	Asymp. Sig. (2-tailed)	.750	

Peak	Treatment	N	Median	Test statistics		Conclusion
	Total	18		Exact Sig. (2-tailed)	.798	
	Water (control)	14	1.809	Mann-Whitney U	19	
TYD1	Chloroform	4	1.924	Asymp. Sig. (2-tailed)	.339	
	Total	18		Exact Sig. (2-tailed)	.382	
	Water (control)	14	1.832	Mann-Whitney U	10	
TYD2	Chloroform	4	2.100	Asymp. Sig. (2-tailed)	.056	
	Total	18		Exact Sig. (2-tailed)	.061	
	Water (control)	14	4.148	Mann-Whitney U	27	
TYE1	Chloroform	4	4.170	Asymp. Sig. (2-tailed)	.915	
	Total	18		Exact Sig. (2-tailed)	.959	
	Water (control)	14	4.094	Mann-Whitney U	25	
TYE2	Chloroform	4	4.147	Asymp. Sig. (2-tailed)	.750	
	Total	18		Exact Sig. (2-tailed)	.798	
	Water (control)	14	0.934	Mann-Whitney U	3	
SMA1	Detergent	3	0.829	Asymp. Sig. (2-tailed)	.023	
	Total	17		Exact Sig. (2-tailed)	.021	
	Water (control)	14	0.897	Mann-Whitney U	6	
SMA2	Detergent	3	0.842	Asymp. Sig. (2-tailed)	.059	
	Total	17		Exact Sig. (2-tailed)	.068	Detergent =
	Water (control)	14	0.393	Mann-Whitney U	0	mixed result
SMB1	Detergent	3	0.604	Asymp. Sig. (2-tailed)	.008	
	Total	17		Exact Sig. (2-tailed)	.003	
	Water (control)	14	0.384	Mann-Whitney U	0	
SMB2	Detergent	2	0.728	Asymp. Sig. (2-tailed)	.026	
	Total	16		Exact Sig. (2-tailed)	.017	
	Water (control)	14	1.818	Mann-Whitney U	0	
TYA1	Detergent	3	1.000	Asymp. Sig. (2-tailed)	.008	
	Total	17		Exact Sig. (2-tailed)	.003	
	Water (control)	14	1.848	Mann-Whitney U	0	
TYA2	Detergent	3	0.971	Asymp. Sig. (2-tailed)	.008	
	Total	17		Exact Sig. (2-tailed)	.003	
	Water (control)	14	2.652	Mann-Whitney U	0	
TYB1	Detergent	3	1.000	Asymp. Sig. (2-tailed)	.008	
	Total	17		Exact Sig. (2-tailed)	.003	
	Water (control)	14	2.669	Mann-Whitney U	0	
TYB2	Detergent	3	1.061	Asymp. Sig. (2-tailed)	.008	
	Total	17		Exact Sig. (2-tailed)	.003	
	Water (control)	14	2.108	Mann-Whitney U	0	
TYC1	Detergent	3	0.816	Asymp. Sig. (2-tailed)	.008	
	Total	17		Exact Sig. (2-tailed)	.003	Detergent =
	Water (control)	14	2.136	Mann-Whitney U	0	unsafe
TYC2	Detergent	4	0.868	Asymp. Sig. (2-tailed)	.003	
	Total	18		Exact Sig. (2-tailed)	.001	
	Water (control)	14	1.809	Mann-Whitney U	0	
TYD1	Detergent	3	0.861	Asymp. Sig. (2-tailed)	.008	
	Total	17		Exact Sig. (2-tailed)	.003	
	Water (control)	14	1.832	Mann-Whitney U	0	
TYD2	Detergent	3	0.729	Asymp. Sig. (2-tailed)	.008	
	Total	17		Exact Sig. (2-tailed)	.003	
	Water (control)	14	4.148	Mann-Whitney U	0	
TYE1	Detergent	3	1.031	Asymp. Sig. (2-tailed)	.008	
	Total	17		Exact Sig. (2-tailed)	.003	
	Water (control)	14	4.094	Mann-Whitney U	0	
TYE2	Detergent	3	1.000	Asymp. Sig. (2-tailed)	.008	
	Total	17		Exact Sig. (2-tailed)	.003	
	Water (control)	14	0.934	Mann-Whitney U	10	EDTA = safe
SMA1	EDTA	2	0.825	Asymp. Sig. (2-tailed)	.525	
	Total	16		Exact Sig. (2-tailed)	.583	
	Water (control)	14	0.897	Mann-Whitney U	5	
SMA2	EDTA	2	0.799	Asymp. Sig. (2-tailed)	.153	
	Total	16		Exact Sig. (2-tailed)	.200	
SMB2	Water (control)	14	0.384	Mann-Whitney U	0	

Peak	Treatment	N	Median	Test statistics		Conclusion
	EDTA	2	0.600	Asymp. Sig. (2-tailed)	.026	
	Total	16		Exact Sig. (2-tailed)	.017	
	Water (control)	14	1.818	Mann-Whitney U	13	
TYA1	EDTA	2	1.570	Asymp. Sig. (2-tailed)	.874	
	Total	16		Exact Sig. (2-tailed)	.933	
	Water (control)	14	1.848	Mann-Whitney U	17	
TYA2	EDTA	3	1.696	Asymp. Sig. (2-tailed)	.614	
	Total	17		Exact Sig. (2-tailed)	.676	
	Water (control)	14	2.652	Mann-Whitney U	0	
TYB1	EDTA	2	1.564	Asymp. Sig. (2-tailed)	.026	
	Total	16		Exact Sig. (2-tailed)	.017	
	Water (control)	14	2.669	Mann-Whitney U	0	
TYB2	EDTA	2	1.711	Asymp. Sig. (2-tailed)	.026	
	Total	16		Exact Sig. (2-tailed)	.017	
	Water (control)	14	2.108	Mann-Whitney U	0	
TYC1	EDTA	2	0.896	Asymp. Sig. (2-tailed)	.026	
	Total	16		Exact Sig. (2-tailed)	.017	EDTA = unsafe
	Water (control)	14	2.136	Mann-Whitney U	0	
TYC2	EDTA	2	1.038	Asymp. Sig. (2-tailed)	.026	
	Total	16		Exact Sig. (2-tailed)	.017	
	Water (control)	14	1.809	Mann-Whitney U	0	
TYD1	EDTA	2	0.676	Asymp. Sig. (2-tailed)	.026	
	Total	16		Exact Sig. (2-tailed)	.017	
	Water (control)	14	1.832	Mann-Whitney U	0	
TYD2	EDTA	3	0.947	Asymp. Sig. (2-tailed)	.008	
	Total	17		Exact Sig. (2-tailed)	.003	
	Water (control)	14	4.148	Mann-Whitney U	0	
TYE1	EDTA	3	0.913	Asymp. Sig. (2-tailed)	.008	
	Total	17		Exact Sig. (2-tailed)	.003	
	Water (control)	14	4.094	Mann-Whitney U	0	
TYE2	EDTA	2	1.301	Asymp. Sig. (2-tailed)	.026	
	Total	16		Exact Sig. (2-tailed)	.017	
	Water (control)	14	0.934	Mann-Whitney U	16	
SMA1	Ethanol	4	0.956	Asymp. Sig. (2-tailed)	.202	
	Total	18		Exact Sig. (2-tailed)	.225	
	Water (control)	14	0.897	Mann-Whitney U	12	
SMA2	Ethanol	4	0.963	Asymp. Sig. (2-tailed)	.089	
	Total	18		Exact Sig. (2-tailed)	.101	Ethanol = safe
	Water (control)	14	0.393	Mann-Whitney U	12	
SMB1	Ethanol	4	0.441	Asymp. Sig. (2-tailed)	.089	
	Total	18		Exact Sig. (2-tailed)	.101	
	Water (control)	14	0.384	Mann-Whitney U	11.5	
SMB2	Ethanol	4	0.419	Asymp. Sig. (2-tailed)	.080	
	Total	18		Exact Sig. (2-tailed)	.082	
	Water (control)	14	1.818	Mann-Whitney U	13	Ethanol =
TYA1	Ethanol	4	1.608	Asymp. Sig. (2-tailed)	.111	unsafe
	Total	18		Exact Sig. (2-tailed)	.127	
	Water (control)	14	1.848	Mann-Whitney U	4	
TYA2	Ethanol	4	1.622	Asymp. Sig. (2-tailed)	.011	
	Total	18		Exact Sig. (2-tailed)	.008	
	Water (control)	14	2.652	Mann-Whitney U	12	
TYB1	Ethanol	4	2.343	Asymp. Sig. (2-tailed)	.089	
	Total	18		Exact Sig. (2-tailed)	.101	
	Water (control)	14	2.669	Mann-Whitney U	4	
TYB2	Ethanol	4	2.339	Asymp. Sig. (2-tailed)	.011	
	Total	18		Exact Sig. (2-tailed)	.008	
	Water (control)	14	2.108	Mann-Whitney U	23	
TYC1	Ethanol	4	1.945	Asymp. Sig. (2-tailed)	.595	
	Total	18		Exact Sig. (2-tailed)	.645	
	Water (control)	14	2.136	Mann-Whitney U	18	
TYC2	Ethanol	4	2.059	Asymp. Sig. (2-tailed)	.288	
	Total	18		Exact Sig. (2-tailed)	.327	

Peak	Treatment	N	Median	Test statistics		Conclusion
TYD1	Water (control)	14	1.809	Mann-Whitney U	19	
	Ethanol	4	1.742	Asymp. Sig. (2-tailed)	.339	
	Total	18		Exact Sig. (2-tailed)	.382	
TYD2	Water (control)	14	1.832	Mann-Whitney U	24	
	Ethanol	4	1.792	Asymp. Sig. (2-tailed)	.671	
	Total	18		Exact Sig. (2-tailed)	.721	
TYE1	Water (control)	14	4.148	Mann-Whitney U	8	
	Ethanol	4	3.540	Asymp. Sig. (2-tailed)	.034	
	Total	18		Exact Sig. (2-tailed)	.035	
TYE2	Water (control)	14	4.094	Mann-Whitney U	8	
	Ethanol	4	3.739	Asymp. Sig. (2-tailed)	.034	
	Total	18		Exact Sig. (2-tailed)	.035	
SMA1	Water (control)	14	0.934	Mann-Whitney U	21	
	Ethyl acetate	4	0.903	Asymp. Sig. (2-tailed)	.457	
	Total	18		Exact Sig. (2-tailed)	.493	
SMA2	Water (control)	14	0.897	Mann-Whitney U	24	
	Ethyl acetate	4	0.892	Asymp. Sig. (2-tailed)	.671	
	Total	18		Exact Sig. (2-tailed)	.721	Ethyl acetate = safe
SMB1	Water (control)	14	0.393	Mann-Whitney U	26	
	Ethyl acetate	4	0.403	Asymp. Sig. (2-tailed)	.832	
	Total	18		Exact Sig. (2-tailed)	.878	
SMB2	Water (control)	14	0.384	Mann-Whitney U	24.5	
	Ethyl acetate	4	0.395	Asymp. Sig. (2-tailed)	.710	
	Total	18		Exact Sig. (2-tailed)	.736	
TYA1	Water (control)	14	1.818	Mann-Whitney U	20	
	Ethyl acetate	4	1.707	Asymp. Sig. (2-tailed)	.396	
	Total	18		Exact Sig. (2-tailed)	.442	
TYA2	Water (control)	14	1.848	Mann-Whitney U	14	
	Ethyl acetate	4	1.735	Asymp. Sig. (2-tailed)	.137	
	Total	18		Exact Sig. (2-tailed)	.158	
TYB1	Water (control)	14	2.652	Mann-Whitney U	18	
	Ethyl acetate	4	2.488	Asymp. Sig. (2-tailed)	.288	
	Total	18		Exact Sig. (2-tailed)	.327	
TYB2	Water (control)	14	2.669	Mann-Whitney U	15	
	Ethyl acetate	4	2.504	Asymp. Sig. (2-tailed)	.167	
	Total	18		Exact Sig. (2-tailed)	.192	
TYC1	Water (control)	14	2.108	Mann-Whitney U	27	
	Ethyl acetate	4	2.047	Asymp. Sig. (2-tailed)	.915	
	Total	18		Exact Sig. (2-tailed)	.959	Ethyl acetate = safe
TYC2	Water (control)	14	2.136	Mann-Whitney U	21	
	Ethyl acetate	4	2.153	Asymp. Sig. (2-tailed)	.457	
	Total	18		Exact Sig. (2-tailed)	.505	
TYD1	Water (control)	14	1.809	Mann-Whitney U	14	
	Ethyl acetate	4	1.997	Asymp. Sig. (2-tailed)	.137	
	Total	18		Exact Sig. (2-tailed)	.158	
TYD2	Water (control)	14	1.832	Mann-Whitney U	22	
	Ethyl acetate	4	1.949	Asymp. Sig. (2-tailed)	.524	
	Total	18		Exact Sig. (2-tailed)	.574	
TYE1	Water (control)	14	4.148	Mann-Whitney U	26	
	Ethyl acetate	4	4.136	Asymp. Sig. (2-tailed)	.832	
	Total	18		Exact Sig. (2-tailed)	.878	
TYE2	Water (control)	14	4.094	Mann-Whitney U	23	
	Ethyl acetate	4	4.260	Asymp. Sig. (2-tailed)	.595	
	Total	18		Exact Sig. (2-tailed)	.645	
SMA1	Water (control)	14	0.934	Mann-Whitney U	0	Gasoline = unsafe
	Gasoline	4	0.672	Asymp. Sig. (2-tailed)	.003	
	Total	18		Exact Sig. (2-tailed)	.001	
SMA2	Water (control)	14	0.897	Mann-Whitney U	0	
	Gasoline	4	0.656	Asymp. Sig. (2-tailed)	.003	
	Total	18		Exact Sig. (2-tailed)	.001	
SMB1	Water (control)	14	0.393	Mann-Whitney U	0	
	Gasoline	4	0.312	Asymp. Sig. (2-tailed)	.003	

Peak	Treatment	N	Median	Test statistics		Conclusion
	Total	18		Exact Sig. (2-tailed)	.001	
	Water (control)	14	0.384	Mann-Whitney U	0	
SMB2	Gasoline	4	0.294	Asymp. Sig. (2-tailed)	.003	
	Total	18		Exact Sig. (2-tailed)	.001	
	Water (control)	14	1.818	Mann-Whitney U	0	
TYA1	Gasoline	4	1.414	Asymp. Sig. (2-tailed)	.003	
	Total	18		Exact Sig. (2-tailed)	.001	
	Water (control)	14	1.848	Mann-Whitney U	0	
TYA2	Gasoline	4	1.440	Asymp. Sig. (2-tailed)	.003	
	Total	18		Exact Sig. (2-tailed)	.001	
	Water (control)	14	2.652	Mann-Whitney U	0	
TYB1	Gasoline	4	2.028	Asymp. Sig. (2-tailed)	.003	
	Total	18		Exact Sig. (2-tailed)	.001	
	Water (control)	14	2.669	Mann-Whitney U	0	
TYB2	Gasoline	4	2.060	Asymp. Sig. (2-tailed)	.003	
	Total	18		Exact Sig. (2-tailed)	.001	
	Water (control)	14	2.108	Mann-Whitney U	0	
TYC1	Gasoline	4	1.712	Asymp. Sig. (2-tailed)	.003	
	Total	18		Exact Sig. (2-tailed)	.001	Gasoline = unsafe
	Water (control)	14	2.136	Mann-Whitney U	0	
TYC2	Gasoline	4	1.732	Asymp. Sig. (2-tailed)	.003	
	Total	18		Exact Sig. (2-tailed)	.001	
	Water (control)	14	1.809	Mann-Whitney U	9	
TYD1	Gasoline	4	1.642	Asymp. Sig. (2-tailed)	.044	
	Total	18		Exact Sig. (2-tailed)	.046	
	Water (control)	14	1.832	Mann-Whitney U	9	
TYD2	Gasoline	4	1.653	Asymp. Sig. (2-tailed)	.044	
	Total	18		Exact Sig. (2-tailed)	.046	
	Water (control)	14	4.148	Mann-Whitney U	8	
TYE1	Gasoline	4	3.373	Asymp. Sig. (2-tailed)	.034	
	Total	18		Exact Sig. (2-tailed)	.035	
	Water (control)	14	4.094	Mann-Whitney U	6	
TYE2	Gasoline	4	3.503	Asymp. Sig. (2-tailed)	.019	
	Total	18		Exact Sig. (2-tailed)	.018	
	Water (control)	14	1.818	Mann-Whitney U	0	Gum arabic = unsafe
TYA1	Gum arabic	4	1.431	Asymp. Sig. (2-tailed)	.003	
	Total	18		Exact Sig. (2-tailed)	.001	
	Water (control)	14	1.848	Mann-Whitney U	0	
TYA2	Gum arabic	2	1.370	Asymp. Sig. (2-tailed)	.026	
	Total	16		Exact Sig. (2-tailed)	.017	
	Water (control)	14	2.652	Mann-Whitney U	0	
TYB1	Gum arabic	4	1.433	Asymp. Sig. (2-tailed)	.003	
	Total	18		Exact Sig. (2-tailed)	.001	
	Water (control)	14	2.669	Mann-Whitney U	0	
TYB2	Gum arabic	2	1.548	Asymp. Sig. (2-tailed)	.026	
	Total	16		Exact Sig. (2-tailed)	.017	
	Water (control)	14	2.108	Mann-Whitney U	0	
TYC1	Gum arabic	4	1.084	Asymp. Sig. (2-tailed)	.003	
	Total	18		Exact Sig. (2-tailed)	.001	
	Water (control)	14	2.136	Mann-Whitney U	0	
TYC2	Gum arabic	2	0.916	Asymp. Sig. (2-tailed)	.026	
	Total	16		Exact Sig. (2-tailed)	.017	
	Water (control)	14	1.809	Mann-Whitney U	0	
TYD1	Gum arabic	4	1.046	Asymp. Sig. (2-tailed)	.003	
	Total	18		Exact Sig. (2-tailed)	.001	
	Water (control)	14	1.832	Mann-Whitney U	0	
TYD2	Gum arabic	2	0.944	Asymp. Sig. (2-tailed)	.026	
	Total	16		Exact Sig. (2-tailed)	.017	
	Water (control)	14	4.148	Mann-Whitney U	0	
TYE1	Gum arabic	4	1.294	Asymp. Sig. (2-tailed)	.003	
	Total	18		Exact Sig. (2-tailed)	.001	
TYE2	Water (control)	14	4.094	Mann-Whitney U	0	

Peak	Treatment	N	Median	Test statistics		Conclusion
	Gum arabic	2	1.217	Asymp. Sig. (2-tailed)	.026	
	Total	16		Exact Sig. (2-tailed)	.017	
SMA1	Water (control)	14	0.934	Mann-Whitney U	0	Hydrogen peroxide = unsafe
	Hydrogen peroxide	4	0.520	Asymp. Sig. (2-tailed)	.003	
	Total	18		Exact Sig. (2-tailed)	.001	
SMA2	Water (control)	14	0.897	Mann-Whitney U	0	
	Hydrogen peroxide	4	0.482	Asymp. Sig. (2-tailed)	.003	
	Total	18		Exact Sig. (2-tailed)	.001	
SMB1	Water (control)	14	0.393	Mann-Whitney U	0	
	Hydrogen peroxide	4	0.158	Asymp. Sig. (2-tailed)	.003	
	Total	18		Exact Sig. (2-tailed)	.001	
SMB2	Water (control)	14	0.384	Mann-Whitney U	0	
	Hydrogen peroxide	4	0.150	Asymp. Sig. (2-tailed)	.003	
	Total	18		Exact Sig. (2-tailed)	.001	
TYA1	Water (control)	14	1.818	Mann-Whitney U	0	Hydrogen peroxide = unsafe
	Hydrogen peroxide	4	1.206	Asymp. Sig. (2-tailed)	.003	
	Total	18		Exact Sig. (2-tailed)	.001	
TYA2	Water (control)	14	1.848	Mann-Whitney U	0	
	Hydrogen peroxide	4	1.225	Asymp. Sig. (2-tailed)	.003	
	Total	18		Exact Sig. (2-tailed)	.001	
TYB1	Water (control)	14	2.652	Mann-Whitney U	0	
	Hydrogen peroxide	4	1.814	Asymp. Sig. (2-tailed)	.003	
	Total	18		Exact Sig. (2-tailed)	.001	
TYB2	Water (control)	14	2.669	Mann-Whitney U	0	
	Hydrogen peroxide	4	1.822	Asymp. Sig. (2-tailed)	.003	
	Total	18		Exact Sig. (2-tailed)	.001	
TYC1	Water (control)	14	2.108	Mann-Whitney U	14	
	Hydrogen peroxide	4	1.730	Asymp. Sig. (2-tailed)	.137	
	Total	18		Exact Sig. (2-tailed)	.158	
TYC2	Water (control)	14	2.136	Mann-Whitney U	3	
	Hydrogen peroxide	4	1.713	Asymp. Sig. (2-tailed)	.008	
	Total	18		Exact Sig. (2-tailed)	.005	
TYD1	Water (control)	14	1.809	Mann-Whitney U	0	
	Hydrogen peroxide	4	1.403	Asymp. Sig. (2-tailed)	.003	
	Total	18		Exact Sig. (2-tailed)	.001	
TYD2	Water (control)	14	1.832	Mann-Whitney U	0	
	Hydrogen peroxide	4	1.346	Asymp. Sig. (2-tailed)	.003	
	Total	18		Exact Sig. (2-tailed)	.001	
TYE1	Water (control)	14	4.148	Mann-Whitney U	4	
	Hydrogen peroxide	4	2.963	Asymp. Sig. (2-tailed)	.011	
	Total	18		Exact Sig. (2-tailed)	.008	
TYE2	Water (control)	14	4.094	Mann-Whitney U	2	
	Hydrogen peroxide	4	2.822	Asymp. Sig. (2-tailed)	.006	
	Total	18		Exact Sig. (2-tailed)	.003	
SMA1	Water (control)	14	0.934	Mann-Whitney U	22	IMS = safe
	IMS	4	0.919	Asymp. Sig. (2-tailed)	.524	
	Total	18		Exact Sig. (2-tailed)	.561	
SMA2	Water (control)	14	0.897	Mann-Whitney U	22	
	IMS	4	0.887	Asymp. Sig. (2-tailed)	.524	
	Total	18		Exact Sig. (2-tailed)	.574	
SMB1	Water (control)	14	0.393	Mann-Whitney U	20	
	IMS	4	0.421	Asymp. Sig. (2-tailed)	.396	
	Total	18		Exact Sig. (2-tailed)	.442	
SMB2	Water (control)	14	0.384	Mann-Whitney U	22.5	
	IMS	4	0.399	Asymp. Sig. (2-tailed)	.559	
	Total	18		Exact Sig. (2-tailed)	.587	
TYA1	Water (control)	14	1.818	Mann-Whitney U	26	IMS = safe
	IMS	4	1.778	Asymp. Sig. (2-tailed)	.832	
	Total	18		Exact Sig. (2-tailed)	.878	
TYA2	Water (control)	14	1.848	Mann-Whitney U	24	
	IMS	4	1.860	Asymp. Sig. (2-tailed)	.671	
	Total	18		Exact Sig. (2-tailed)	.721	

Peak	Treatment	N	Median	Test statistics		Conclusion
TYB1	Water (control)	14	2.652	Mann-Whitney U	26	
	IMS	4	2.563	Asymp. Sig. (2-tailed)	.832	
	Total	18		Exact Sig. (2-tailed)	.862	
TYB2	Water (control)	14	2.669	Mann-Whitney U	26	
	IMS	4	2.645	Asymp. Sig. (2-tailed)	.832	
	Total	18		Exact Sig. (2-tailed)	.878	
TYC1	Water (control)	14	2.108	Mann-Whitney U	15	
	IMS	4	2.184	Asymp. Sig. (2-tailed)	.167	
	Total	18		Exact Sig. (2-tailed)	.192	
TYC2	Water (control)	14	2.136	Mann-Whitney U	17	
	IMS	4	2.336	Asymp. Sig. (2-tailed)	.243	
	Total	18		Exact Sig. (2-tailed)	.277	
TYD1	Water (control)	14	1.809	Mann-Whitney U	8	
	IMS	4	2.088	Asymp. Sig. (2-tailed)	.034	
	Total	18		Exact Sig. (2-tailed)	.035	
TYD2	Water (control)	14	1.832	Mann-Whitney U	8	
	IMS	4	2.146	Asymp. Sig. (2-tailed)	.034	
	Total	18		Exact Sig. (2-tailed)	.035	
TYE1	Water (control)	14	4.148	Mann-Whitney U	26	
	IMS	4	4.171	Asymp. Sig. (2-tailed)	.832	
	Total	18		Exact Sig. (2-tailed)	.878	
TYE2	Water (control)	14	4.094	Mann-Whitney U	20	
	IMS	4	4.379	Asymp. Sig. (2-tailed)	.396	
	Total	18		Exact Sig. (2-tailed)	.442	
SMA1	Water (control)	14	0.934	Mann-Whitney U	6	
	Kerosene	4	0.880	Asymp. Sig. (2-tailed)	.019	
	Total	18		Exact Sig. (2-tailed)	.017	
SMA2	Water (control)	14	0.897	Mann-Whitney U	4.5	
	Kerosene	4	0.812	Asymp. Sig. (2-tailed)	.013	
	Total	18		Exact Sig. (2-tailed)	.008	Kerosene = unsafe
SMB1	Water (control)	14	0.393	Mann-Whitney U	21.5	
	Kerosene	4	0.389	Asymp. Sig. (2-tailed)	.490	
	Total	18		Exact Sig. (2-tailed)	.517	
SMB2	Water (control)	14	0.384	Mann-Whitney U	13	
	Kerosene	4	0.367	Asymp. Sig. (2-tailed)	.111	
	Total	18		Exact Sig. (2-tailed)	.127	
TYA1	Water (control)	14	1.818	Mann-Whitney U	11	Kerosene = unsafe
	Kerosene	4	1.626	Asymp. Sig. (2-tailed)	.071	
	Total	18		Exact Sig. (2-tailed)	.079	
TYA2	Water (control)	14	1.848	Mann-Whitney U	13	
	Kerosene	4	1.711	Asymp. Sig. (2-tailed)	.111	
	Total	18		Exact Sig. (2-tailed)	.127	
TYB1	Water (control)	14	2.652	Mann-Whitney U	9	
	Kerosene	4	2.327	Asymp. Sig. (2-tailed)	.044	
	Total	18		Exact Sig. (2-tailed)	.046	
TYB2	Water (control)	14	2.669	Mann-Whitney U	9	
	Kerosene	4	2.415	Asymp. Sig. (2-tailed)	.044	
	Total	18		Exact Sig. (2-tailed)	.046	
TYC1	Water (control)	14	2.108	Mann-Whitney U	14	
	Kerosene	4	1.973	Asymp. Sig. (2-tailed)	.137	
	Total	18		Exact Sig. (2-tailed)	.158	
TYC2	Water (control)	14	2.136	Mann-Whitney U	16	
	Kerosene	4	2.062	Asymp. Sig. (2-tailed)	.203	
	Total	18		Exact Sig. (2-tailed)	.233	
TYD1	Water (control)	14	1.809	Mann-Whitney U	26	
	Kerosene	4	1.828	Asymp. Sig. (2-tailed)	.832	
	Total	18		Exact Sig. (2-tailed)	.878	
TYD2	Water (control)	14	1.832	Mann-Whitney U	25	
	Kerosene	4	1.857	Asymp. Sig. (2-tailed)	.750	
	Total	18		Exact Sig. (2-tailed)	.798	
TYE1	Water (control)	14	4.148	Mann-Whitney U	17	
	Kerosene	4	3.798	Asymp. Sig. (2-tailed)	.243	

Peak	Treatment	N	Median	Test statistics		Conclusion
	Total	18		Exact Sig. (2-tailed)	.277	
	Water (control)	14	4.094	Mann-Whitney U	21	
TYE2	Kerosene	4	3.970	Asymp. Sig. (2-tailed)	.457	
	Total	18		Exact Sig. (2-tailed)	.505	
	Water (control)	14	0.934	Mann-Whitney U	0	
SMA1	Linseed oil	4	0.613	Asymp. Sig. (2-tailed)	.003	
	Total	18		Exact Sig. (2-tailed)	.001	
	Water (control)	14	0.897	Mann-Whitney U	0	
SMA2	Linseed oil	4	0.548	Asymp. Sig. (2-tailed)	.003	
	Total	18		Exact Sig. (2-tailed)	.001	Linseed oil =
	Water (control)	14	0.393	Mann-Whitney U	0	unsafe
SMB1	Linseed oil	4	0.220	Asymp. Sig. (2-tailed)	.003	
	Total	18		Exact Sig. (2-tailed)	.001	
	Water (control)	14	0.384	Mann-Whitney U	0	
SMB2	Linseed oil	4	0.220	Asymp. Sig. (2-tailed)	.003	
	Total	18		Exact Sig. (2-tailed)	.001	
	Water (control)	14	1.818	Mann-Whitney U	4	
TYA1	Linseed oil	4	1.467	Asymp. Sig. (2-tailed)	.011	
	Total	18		Exact Sig. (2-tailed)	.008	
	Water (control)	14	1.848	Mann-Whitney U	2	
TYA2	Linseed oil	4	1.512	Asymp. Sig. (2-tailed)	.006	
	Total	18		Exact Sig. (2-tailed)	.003	
	Water (control)	14	2.652	Mann-Whitney U	3	
TYB1	Linseed oil	4	2.222	Asymp. Sig. (2-tailed)	.008	
	Total	18		Exact Sig. (2-tailed)	.005	
	Water (control)	14	2.669	Mann-Whitney U	1	
TYB2	Linseed oil	4	2.196	Asymp. Sig. (2-tailed)	.004	
	Total	18		Exact Sig. (2-tailed)	.001	
	Water (control)	14	2.108	Mann-Whitney U	4	
TYC1	Linseed oil	4	1.768	Asymp. Sig. (2-tailed)	.011	
	Total	18		Exact Sig. (2-tailed)	.008	Linseed oil =
	Water (control)	14	2.136	Mann-Whitney U	1	unsafe
TYC2	Linseed oil	4	1.815	Asymp. Sig. (2-tailed)	.004	
	Total	18		Exact Sig. (2-tailed)	.001	
	Water (control)	14	1.809	Mann-Whitney U	17	
TYD1	Linseed oil	4	1.709	Asymp. Sig. (2-tailed)	.243	
	Total	18		Exact Sig. (2-tailed)	.277	
	Water (control)	14	1.832	Mann-Whitney U	12	
TYD2	Linseed oil	4	1.694	Asymp. Sig. (2-tailed)	.089	
	Total	18		Exact Sig. (2-tailed)	.101	
	Water (control)	14	4.148	Mann-Whitney U	19	
TYE1	Linseed oil	4	4.429	Asymp. Sig. (2-tailed)	.339	
	Total	18		Exact Sig. (2-tailed)	.382	
	Water (control)	14	4.094	Mann-Whitney U	17	
TYE2	Linseed oil	4	4.412	Asymp. Sig. (2-tailed)	.243	
	Total	18		Exact Sig. (2-tailed)	.277	
	Water (control)	14	1.818	Mann-Whitney U	0	Mercury (II)
TYA1	Mercury (II) chloride	2	0.813	Asymp. Sig. (2-tailed)	.026	chloride =
	Total	16		Exact Sig. (2-tailed)	.017	unsafe
	Water (control)	14	1.848	Mann-Whitney U	11	
TYA2	Mercury (II) chloride	4	1.297	Asymp. Sig. (2-tailed)	.071	
	Total	18		Exact Sig. (2-tailed)	.079	
	Water (control)	14	2.669	Mann-Whitney U	4	
TYB2	Mercury (II) chloride	4	1.421	Asymp. Sig. (2-tailed)	.011	
	Total	18		Exact Sig. (2-tailed)	.008	
	Water (control)	14	2.108	Mann-Whitney U	0	
TYC1	Mercury (II) chloride	2	0.557	Asymp. Sig. (2-tailed)	.026	
	Total	16		Exact Sig. (2-tailed)	.017	
	Water (control)	14	2.136	Mann-Whitney U	0	
TYC2	Mercury (II) chloride	2	0.549	Asymp. Sig. (2-tailed)	.026	
	Total	16		Exact Sig. (2-tailed)	.017	
TYD1	Water (control)	14	1.809	Mann-Whitney U	0	

Peak	Treatment	N	Median	Test statistics		Conclusion
	Mercury (II) chloride	2	0.507	Asymp. Sig. (2-tailed)	.026	
	Total	16		Exact Sig. (2-tailed)	.017	
TYE1	Water (control)	14	4.148	Mann-Whitney U	0	
	Mercury (II) chloride	2	0.570	Asymp. Sig. (2-tailed)	.026	
	Total	16		Exact Sig. (2-tailed)	.017	
TYE2	Water (control)	14	4.094	Mann-Whitney U	0	
	Mercury (II) chloride	2	0.735	Asymp. Sig. (2-tailed)	.026	
	Total	16		Exact Sig. (2-tailed)	.017	
SMA1	Water (control)	14	0.934	Mann-Whitney U	15	
	Methylmethacrylate/ethylacrylate	4	0.830	Asymp. Sig. (2-tailed)	.167	
	Total	18		Exact Sig. (2-tailed)	.185	
SMA2	Water (control)	14	0.897	Mann-Whitney U	19.5	
	Methylmethacrylate/ethylacrylate	4	0.829	Asymp. Sig. (2-tailed)	.366	Methyl-
	Total	18		Exact Sig. (2-tailed)	.393	methacrylate/
SMB1	Water (control)	14	0.393	Mann-Whitney U	15	ethylacrylate =
	Methylmethacrylate/ethylacrylate	4	0.364	Asymp. Sig. (2-tailed)	.167	safe
	Total	18		Exact Sig. (2-tailed)	.192	
SMB2	Water (control)	14	0.384	Mann-Whitney U	21	
	Methylmethacrylate/ethylacrylate	4	0.359	Asymp. Sig. (2-tailed)	.457	
	Total	18		Exact Sig. (2-tailed)	.505	
TYA1	Water (control)	14	1.818	Mann-Whitney U	18	
	Methylmethacrylate/ethylacrylate	4	1.616	Asymp. Sig. (2-tailed)	.288	
	Total	18		Exact Sig. (2-tailed)	.327	
TYA2	Water (control)	14	1.848	Mann-Whitney U	15	
	Methylmethacrylate/ethylacrylate	4	1.621	Asymp. Sig. (2-tailed)	.167	
	Total	18		Exact Sig. (2-tailed)	.192	
TYB1	Water (control)	14	2.652	Mann-Whitney U	18	
	Methylmethacrylate/ethylacrylate	4	2.352	Asymp. Sig. (2-tailed)	.288	
	Total	18		Exact Sig. (2-tailed)	.327	
TYB2	Water (control)	14	2.669	Mann-Whitney U	15	
	Methylmethacrylate/ethylacrylate	4	2.352	Asymp. Sig. (2-tailed)	.167	
	Total	18		Exact Sig. (2-tailed)	.192	
TYC1	Water (control)	14	2.108	Mann-Whitney U	22	
	Methylmethacrylate/ethylacrylate	4	2.032	Asymp. Sig. (2-tailed)	.524	Methyl-
	Total	18		Exact Sig. (2-tailed)	.574	methacrylate/
TYC2	Water (control)	14	2.136	Mann-Whitney U	19	ethylacrylate =
	Methylmethacrylate/ethylacrylate	4	2.024	Asymp. Sig. (2-tailed)	.339	unsafe
	Total	18		Exact Sig. (2-tailed)	.382	
TYD1	Water (control)	14	1.809	Mann-Whitney U	21	
	Methylmethacrylate/ethylacrylate	4	1.747	Asymp. Sig. (2-tailed)	.457	
	Total	18		Exact Sig. (2-tailed)	.505	
TYD2	Water (control)	14	1.832	Mann-Whitney U	14	
	Methylmethacrylate/ethylacrylate	4	1.716	Asymp. Sig. (2-tailed)	.137	
	Total	18		Exact Sig. (2-tailed)	.158	
TYE1	Water (control)	14	4.148	Mann-Whitney U	4	
	Methylmethacrylate/ethylacrylate	4	3.358	Asymp. Sig. (2-tailed)	.011	
	Total	18		Exact Sig. (2-tailed)	.008	
TYE2	Water (control)	14	4.094	Mann-Whitney U	5	
	Methylmethacrylate/ethylacrylate	4	3.298	Asymp. Sig. (2-tailed)	.015	
	Total	18		Exact Sig. (2-tailed)	.012	
SMA1	Water (control)	14	0.934	Mann-Whitney U	0	
	Mineral oil	16	0.233	Asymp. Sig. (2-tailed)	.000	
	Total	30		Exact Sig. (2-tailed)	.000	
SMA2	Water (control)	14	0.897	Mann-Whitney U	0	
	Mineral oil	16	0.200	Asymp. Sig. (2-tailed)	.000	
	Total	30		Exact Sig. (2-tailed)	.000	Mineral oil =
SMB1	Water (control)	14	0.393	Mann-Whitney U	0	unsafe
	Mineral oil	15	0.096	Asymp. Sig. (2-tailed)	.000	
	Total	29		Exact Sig. (2-tailed)	.000	
SMB2	Water (control)	14	0.384	Mann-Whitney U	0	
	Mineral oil	16	0.068	Asymp. Sig. (2-tailed)	.000	
	Total	30		Exact Sig. (2-tailed)	.000	

Peak	Treatment	N	Median	Test statistics		Conclusion
TYA1	Water (control)	14	1.818	Mann-Whitney U	0	Mineral oil = unsafe
	Mineral oil	16	0.478	Asymp. Sig. (2-tailed)	.000	
	Total	30		Exact Sig. (2-tailed)	.000	
TYA2	Water (control)	14	1.848	Mann-Whitney U	0	
	Mineral oil	16	0.418	Asymp. Sig. (2-tailed)	.000	
	Total	30		Exact Sig. (2-tailed)	.000	
TYB1	Water (control)	14	2.652	Mann-Whitney U	0	
	Mineral oil	16	0.676	Asymp. Sig. (2-tailed)	.000	
	Total	30		Exact Sig. (2-tailed)	.000	
TYB2	Water (control)	14	2.669	Mann-Whitney U	0	
	Mineral oil	16	0.596	Asymp. Sig. (2-tailed)	.000	
	Total	30		Exact Sig. (2-tailed)	.000	
TYC1	Water (control)	14	2.108	Mann-Whitney U	0	
	Mineral oil	16	0.469	Asymp. Sig. (2-tailed)	.000	
	Total	30		Exact Sig. (2-tailed)	.000	
TYC2	Water (control)	14	2.136	Mann-Whitney U	0	
	Mineral oil	16	0.467	Asymp. Sig. (2-tailed)	.000	
	Total	30		Exact Sig. (2-tailed)	.000	
TYD1	Water (control)	14	1.809	Mann-Whitney U	0	
	Mineral oil	16	0.423	Asymp. Sig. (2-tailed)	.000	
	Total	30		Exact Sig. (2-tailed)	.000	
TYD2	Water (control)	14	1.832	Mann-Whitney U	0	
	Mineral oil	16	0.425	Asymp. Sig. (2-tailed)	.000	
	Total	30		Exact Sig. (2-tailed)	.000	
TYE1	Water (control)	14	4.148	Mann-Whitney U	0	
	Mineral oil	16	0.945	Asymp. Sig. (2-tailed)	.000	
	Total	30		Exact Sig. (2-tailed)	.000	
TYE2	Water (control)	14	4.094	Mann-Whitney U	0	
	Mineral oil	16	0.925	Asymp. Sig. (2-tailed)	.000	
	Total	30		Exact Sig. (2-tailed)	.000	
SMA1	Water (control)	14	0.934	Mann-Whitney U	0	Pepsin = unsafe
	Pepsin	4	0.274	Asymp. Sig. (2-tailed)	.003	
	Total	18		Exact Sig. (2-tailed)	.001	
SMA2	Water (control)	14	0.897	Mann-Whitney U	0	
	Pepsin	4	0.246	Asymp. Sig. (2-tailed)	.003	
	Total	18		Exact Sig. (2-tailed)	.001	
SMB1	Water (control)	14	0.393	Mann-Whitney U	0	
	Pepsin	4	0.165	Asymp. Sig. (2-tailed)	.003	
	Total	18		Exact Sig. (2-tailed)	.001	
SMB2	Water (control)	14	0.384	Mann-Whitney U	0	
	Pepsin	4	0.168	Asymp. Sig. (2-tailed)	.003	
	Total	18		Exact Sig. (2-tailed)	.001	
TYA1	Water (control)	14	1.818	Mann-Whitney U	0	Pepsin = unsafe
	Pepsin	4	0.622	Asymp. Sig. (2-tailed)	.003	
	Total	18		Exact Sig. (2-tailed)	.001	
TYA2	Water (control)	14	1.848	Mann-Whitney U	0	
	Pepsin	4	0.653	Asymp. Sig. (2-tailed)	.003	
	Total	18		Exact Sig. (2-tailed)	.001	
TYB1	Water (control)	14	2.652	Mann-Whitney U	0	
	Pepsin	4	0.874	Asymp. Sig. (2-tailed)	.003	
	Total	18		Exact Sig. (2-tailed)	.001	
TYB2	Water (control)	14	2.669	Mann-Whitney U	0	
	Pepsin	4	0.896	Asymp. Sig. (2-tailed)	.003	
	Total	18		Exact Sig. (2-tailed)	.001	
TYC1	Water (control)	14	2.108	Mann-Whitney U	0	
	Pepsin	4	0.424	Asymp. Sig. (2-tailed)	.003	
	Total	18		Exact Sig. (2-tailed)	.001	
TYC2	Water (control)	14	2.136	Mann-Whitney U	0	
	Pepsin	4	0.419	Asymp. Sig. (2-tailed)	.003	
	Total	18		Exact Sig. (2-tailed)	.001	
TYD1	Water (control)	14	1.809	Mann-Whitney U	0	
	Pepsin	4	0.415	Asymp. Sig. (2-tailed)	.003	

Peak	Treatment	N	Median	Test statistics		Conclusion
	Total	18		Exact Sig. (2-tailed)	.001	
TYD2	Water (control)	14	1.832	Mann-Whitney U	0	
	Pepsin	4	0.412	Asymp. Sig. (2-tailed)	.003	
	Total	18		Exact Sig. (2-tailed)	.001	
TYE1	Water (control)	14	4.148	Mann-Whitney U	0	
	Pepsin	4	0.569	Asymp. Sig. (2-tailed)	.003	
	Total	18		Exact Sig. (2-tailed)	.001	
TYE2	Water (control)	14	4.094	Mann-Whitney U	0	
	Pepsin	4	0.581	Asymp. Sig. (2-tailed)	.003	
	Total	18		Exact Sig. (2-tailed)	.001	
TYA1	Water (control)	14	1.818	Mann-Whitney U	0	Pepsin heated to 37°C for 48 hours = unsafe
	Pepsin - 48H @ 37°C	4	0.594	Asymp. Sig. (2-tailed)	.003	
	Total	18		Exact Sig. (2-tailed)	.001	
SMA1	Water (control)	14	0.934	Mann-Whitney U	26	Poly(vinyl) butyral resin = safe
	Poly(vinyl) butyral resin	4	0.947	Asymp. Sig. (2-tailed)	.832	
	Total	18		Exact Sig. (2-tailed)	.862	
SMA2	Water (control)	14	0.897	Mann-Whitney U	23	
	Poly(vinyl) butyral resin	4	0.915	Asymp. Sig. (2-tailed)	.595	
	Total	18		Exact Sig. (2-tailed)	.645	
SMB1	Water (control)	14	0.393	Mann-Whitney U	9	
	Poly(vinyl) butyral resin	4	0.479	Asymp. Sig. (2-tailed)	.044	
	Total	18		Exact Sig. (2-tailed)	.046	
SMB2	Water (control)	14	0.384	Mann-Whitney U	12	
	Poly(vinyl) butyral resin	4	0.421	Asymp. Sig. (2-tailed)	.089	
	Total	18		Exact Sig. (2-tailed)	.101	
TYA1	Water (control)	14	1.818	Mann-Whitney U	3	Poly(vinyl) butyral resin = unsafe
	Poly(vinyl) butyral resin	4	1.383	Asymp. Sig. (2-tailed)	.008	
	Total	18		Exact Sig. (2-tailed)	.005	
TYA2	Water (control)	14	1.848	Mann-Whitney U	1	
	Poly(vinyl) butyral resin	4	1.486	Asymp. Sig. (2-tailed)	.004	
	Total	18		Exact Sig. (2-tailed)	.001	
TYB1	Water (control)	14	2.652	Mann-Whitney U	4	
	Poly(vinyl) butyral resin	4	1.929	Asymp. Sig. (2-tailed)	.011	
	Total	18		Exact Sig. (2-tailed)	.008	
TYB2	Water (control)	14	2.669	Mann-Whitney U	1	
	Poly(vinyl) butyral resin	4	2.079	Asymp. Sig. (2-tailed)	.004	
	Total	18		Exact Sig. (2-tailed)	.001	
TYC1	Water (control)	14	2.108	Mann-Whitney U	6	
	Poly(vinyl) butyral resin	4	1.661	Asymp. Sig. (2-tailed)	.019	
	Total	18		Exact Sig. (2-tailed)	.018	
TYC2	Water (control)	14	2.136	Mann-Whitney U	3	
	Poly(vinyl) butyral resin	4	1.710	Asymp. Sig. (2-tailed)	.008	
	Total	18		Exact Sig. (2-tailed)	.005	
TYD1	Water (control)	14	1.809	Mann-Whitney U	12	
	Poly(vinyl) butyral resin	4	1.595	Asymp. Sig. (2-tailed)	.089	
	Total	18		Exact Sig. (2-tailed)	.101	
TYD2	Water (control)	14	1.832	Mann-Whitney U	10	
	Poly(vinyl) butyral resin	4	1.654	Asymp. Sig. (2-tailed)	.056	
	Total	18		Exact Sig. (2-tailed)	.061	
TYE1	Water (control)	14	4.148	Mann-Whitney U	3	
	Poly(vinyl) butyral resin	4	2.912	Asymp. Sig. (2-tailed)	.008	
	Total	18		Exact Sig. (2-tailed)	.005	
TYE2	Water (control)	14	4.094	Mann-Whitney U	4	
	Poly(vinyl) butyral resin	4	3.267	Asymp. Sig. (2-tailed)	.011	
	Total	18		Exact Sig. (2-tailed)	.008	
SMA1	Water (control)	14	0.934	Mann-Whitney U	0	Potassium carbonate = preserving
	Potassium carbonate	4	1.596	Asymp. Sig. (2-tailed)	.003	
	Total	18		Exact Sig. (2-tailed)	.000	
SMA2	Water (control)	14	0.897	Mann-Whitney U	0	
	Potassium carbonate	4	1.660	Asymp. Sig. (2-tailed)	.003	
	Total	18		Exact Sig. (2-tailed)	.001	
SMB1	Water (control)	14	0.393	Mann-Whitney U	0	

Peak	Treatment	N	Median	Test statistics		Conclusion
	Potassium carbonate	4	0.912	Asymp. Sig. (2-tailed)	.003	
	Total	18		Exact Sig. (2-tailed)	.001	
	Water (control)	14	0.384	Mann-Whitney U	0	
SMB2	Potassium carbonate	4	0.921	Asymp. Sig. (2-tailed)	.003	
	Total	18		Exact Sig. (2-tailed)	.001	
	Water (control)	14	1.818	Mann-Whitney U	16	
TYA1	Potassium carbonate	4	1.596	Asymp. Sig. (2-tailed)	.203	
	Total	18		Exact Sig. (2-tailed)	.233	
	Water (control)	14	1.848	Mann-Whitney U	24	
TYA2	Potassium carbonate	4	1.868	Asymp. Sig. (2-tailed)	.671	
	Total	18		Exact Sig. (2-tailed)	.721	
	Water (control)	14	2.652	Mann-Whitney U	7	
TYB1	Potassium carbonate	3	2.306	Asymp. Sig. (2-tailed)	.078	
	Total	17		Exact Sig. (2-tailed)	.091	
	Water (control)	14	2.669	Mann-Whitney U	11	
TYB2	Potassium carbonate	4	2.139	Asymp. Sig. (2-tailed)	.071	
	Total	18		Exact Sig. (2-tailed)	.079	
	Water (control)	14	2.108	Mann-Whitney U	0	
TYC1	Potassium carbonate	4	1.090	Asymp. Sig. (2-tailed)	.003	
	Total	18		Exact Sig. (2-tailed)	.001	Potassium carbonate = unsafe
	Water (control)	14	2.136	Mann-Whitney U	0	
TYC2	Potassium carbonate	4	1.118	Asymp. Sig. (2-tailed)	.003	
	Total	18		Exact Sig. (2-tailed)	.001	
	Water (control)	14	1.809	Mann-Whitney U	0	
TYD1	Potassium carbonate	3	1.108	Asymp. Sig. (2-tailed)	.008	
	Total	17		Exact Sig. (2-tailed)	.003	
	Water (control)	14	1.832	Mann-Whitney U	0	
TYD2	Potassium carbonate	4	1.086	Asymp. Sig. (2-tailed)	.003	
	Total	18		Exact Sig. (2-tailed)	.001	
	Water (control)	14	4.148	Mann-Whitney U	0	
TYE1	Potassium carbonate	4	1.578	Asymp. Sig. (2-tailed)	.003	
	Total	18		Exact Sig. (2-tailed)	.001	
	Water (control)	14	4.094	Mann-Whitney U	0	
TYE2	Potassium carbonate	4	1.587	Asymp. Sig. (2-tailed)	.003	
	Total	18		Exact Sig. (2-tailed)	.001	
	Water (control)	14	0.934	Mann-Whitney U	0	
SMA1	Potassium carbonate - 6H @ 80°C	3	0.231	Asymp. Sig. (2-tailed)	.008	
	Total	17		Exact Sig. (2-tailed)	.003	Heating potassium carbonate to 80°C for 6 hours = unsafe
	Water (control)	14	0.897	Mann-Whitney U	0	
SMA2	Potassium carbonate - 6H @ 80°C	2	0.211	Asymp. Sig. (2-tailed)	.026	
	Total	16		Exact Sig. (2-tailed)	.017	
	Water (control)	14	0.393	Mann-Whitney U	0	
SMB1	Potassium carbonate - 6H @ 80°C	2	0.188	Asymp. Sig. (2-tailed)	.026	
	Total	16		Exact Sig. (2-tailed)	.017	
	Water (control)	14	1.818	Mann-Whitney U	0	
TYA1	Potassium carbonate - 6H @ 80°C	4	0.487	Asymp. Sig. (2-tailed)	.003	
	Total	18		Exact Sig. (2-tailed)	.001	
	Water (control)	14	2.652	Mann-Whitney U	0	
TYB1	Potassium carbonate - 6H @ 80°C	2	0.721	Asymp. Sig. (2-tailed)	.026	
	Total	16		Exact Sig. (2-tailed)	.017	
	Water (control)	14	2.108	Mann-Whitney U	0	Heating potassium carbonate to 80°C for 6 hours = unsafe
TYC1	Potassium carbonate - 6H @ 80°C	2	0.342	Asymp. Sig. (2-tailed)	.026	
	Total	16		Exact Sig. (2-tailed)	.017	
	Water (control)	14	1.809	Mann-Whitney U	0	
TYD1	Potassium carbonate - 6H @ 80°C	2	0.342	Asymp. Sig. (2-tailed)	.026	
	Total	16		Exact Sig. (2-tailed)	.017	
	Water (control)	14	4.148	Mann-Whitney U	0	
TYE1	Potassium carbonate - 6H @ 80°C	2	0.526	Asymp. Sig. (2-tailed)	.026	
	Total	16		Exact Sig. (2-tailed)	.017	
	Water (control)	14	0.934	Mann-Whitney U	20	PVAC = safe
SMA1	PVAC	4	0.985	Asymp. Sig. (2-tailed)	.395	
	Total	18		Exact Sig. (2-tailed)	.431	

Peak	Treatment	N	Median	Test statistics		Conclusion
SMA2	Water (control)	14	0.897	Mann-Whitney U	21	
	PVAC	4	0.934	Asymp. Sig. (2-tailed)	.457	
	Total	18		Exact Sig. (2-tailed)	.505	
SMB1	Water (control)	14	0.393	Mann-Whitney U	8	
	PVAC	4	0.445	Asymp. Sig. (2-tailed)	.034	
	Total	18		Exact Sig. (2-tailed)	.035	
SMB2	Water (control)	14	0.384	Mann-Whitney U	17.5	
	PVAC	4	0.416	Asymp. Sig. (2-tailed)	.265	
	Total	18		Exact Sig. (2-tailed)	.286	
TYA1	Water (control)	14	1.818	Mann-Whitney U	5	
	PVAC	4	1.532	Asymp. Sig. (2-tailed)	.015	
	Total	18		Exact Sig. (2-tailed)	.012	
TYA2	Water (control)	14	1.848	Mann-Whitney U	3	
	PVAC	4	1.551	Asymp. Sig. (2-tailed)	.008	
	Total	18		Exact Sig. (2-tailed)	.005	
TYB1	Water (control)	14	2.652	Mann-Whitney U	2	
	PVAC	4	2.206	Asymp. Sig. (2-tailed)	.006	
	Total	18		Exact Sig. (2-tailed)	.003	
TYB2	Water (control)	14	2.669	Mann-Whitney U	1	
	PVAC	4	2.265	Asymp. Sig. (2-tailed)	.004	
	Total	18		Exact Sig. (2-tailed)	.001	
TYC1	Water (control)	14	2.108	Mann-Whitney U	0	
	PVAC	4	1.780	Asymp. Sig. (2-tailed)	.003	
	Total	18		Exact Sig. (2-tailed)	.001	PVAC = unsafe
TYC2	Water (control)	14	2.136	Mann-Whitney U	2	
	PVAC	4	1.817	Asymp. Sig. (2-tailed)	.006	
	Total	18		Exact Sig. (2-tailed)	.003	
TYD1	Water (control)	14	1.809	Mann-Whitney U	18.5	
	PVAC	4	1.734	Asymp. Sig. (2-tailed)	.313	
	Total	18		Exact Sig. (2-tailed)	.337	
TYD2	Water (control)	14	1.832	Mann-Whitney U	22	
	PVAC	4	1.872	Asymp. Sig. (2-tailed)	.524	
	Total	18		Exact Sig. (2-tailed)	.574	
TYE1	Water (control)	14	4.148	Mann-Whitney U	12	
	PVAC	4	3.725	Asymp. Sig. (2-tailed)	.089	
	Total	18		Exact Sig. (2-tailed)	.101	
TYE2	Water (control)	14	4.094	Mann-Whitney U	22	
	PVAC	4	4.044	Asymp. Sig. (2-tailed)	.524	
	Total	18		Exact Sig. (2-tailed)	.574	
SMA1	Water (control)	14	0.934	Mann-Whitney U	5	
	PVAC/PVAL	4	0.842	Asymp. Sig. (2-tailed)	.015	
	Total	18		Exact Sig. (2-tailed)	.011	
SMA2	Water (control)	14	0.897	Mann-Whitney U	11.5	
	PVAC/PVAL	4	0.850	Asymp. Sig. (2-tailed)	.080	
	Total	18		Exact Sig. (2-tailed)	.082	PVAC/PVAL =
SMB1	Water (control)	14	0.393	Mann-Whitney U	2.5	unsafe
	PVAC/PVAL	4	0.387	Asymp. Sig. (2-tailed)	.426	
	Total	18		Exact Sig. (2-tailed)	.454	
SMB2	Water (control)	14	0.384	Mann-Whitney U	21	
	PVAC/PVAL	4	0.381	Asymp. Sig. (2-tailed)	.457	
	Total	18		Exact Sig. (2-tailed)	.505	
TYA1	Water (control)	14	1.818	Mann-Whitney U	0	PVAC/PVAL =
	PVAC/PVAL	4	1.204	Asymp. Sig. (2-tailed)	.003	unsafe
	Total	18		Exact Sig. (2-tailed)	.001	
TYA2	Water (control)	14	1.848	Mann-Whitney U	0	
	PVAC/PVAL	4	1.322	Asymp. Sig. (2-tailed)	.003	
	Total	18		Exact Sig. (2-tailed)	.001	
TYB1	Water (control)	14	2.652	Mann-Whitney U	0	
	PVAC/PVAL	4	1.705	Asymp. Sig. (2-tailed)	.003	
	Total	18		Exact Sig. (2-tailed)	.001	
TYB2	Water (control)	14	2.669	Mann-Whitney U	0	
	PVAC/PVAL	4	1.861	Asymp. Sig. (2-tailed)	.003	

Peak	Treatment	N	Median	Test statistics		Conclusion
	Total	18		Exact Sig. (2-tailed)	.001	
TYC1	Water (control)	14	2.108	Mann-Whitney U	0	
	PVAC/PVAL	4	1.422	Asymp. Sig. (2-tailed)	.003	
	Total	18		Exact Sig. (2-tailed)	.001	
TYC2	Water (control)	14	2.136	Mann-Whitney U	0	
	PVAC/PVAL	4	1.551	Asymp. Sig. (2-tailed)	.003	
	Total	18		Exact Sig. (2-tailed)	.001	
TYD1	Water (control)	14	1.809	Mann-Whitney U	0	
	PVAC/PVAL	4	1.400	Asymp. Sig. (2-tailed)	.003	
	Total	18		Exact Sig. (2-tailed)	.001	
TYD2	Water (control)	14	1.832	Mann-Whitney U	0	
	PVAC/PVAL	4	1.551	Asymp. Sig. (2-tailed)	.003	
	Total	18		Exact Sig. (2-tailed)	.001	
TYE1	Water (control)	14	4.148	Mann-Whitney U	4	
	PVAC/PVAL	4	3.238	Asymp. Sig. (2-tailed)	.011	
	Total	18		Exact Sig. (2-tailed)	.008	
TYE2	Water (control)	14	4.094	Mann-Whitney U	6	
	PVAC/PVAL	4	3.276	Asymp. Sig. (2-tailed)	.019	
	Total	18		Exact Sig. (2-tailed)	.018	
SMA1	Water (control)	14	0.934	Mann-Whitney U	5	Shellac = safe
	Shellac	2	1.136	Asymp. Sig. (2-tailed)	.153	
	Total	16		Exact Sig. (2-tailed)	.200	
SMA2	Water (control)	14	0.897	Mann-Whitney U	13	
	Shellac	2	0.959	Asymp. Sig. (2-tailed)	.874	
	Total	16		Exact Sig. (2-tailed)	.933	
SMB1	Water (control)	14	0.393	Mann-Whitney U	0	
	Shellac	2	0.637	Asymp. Sig. (2-tailed)	.026	
	Total	16		Exact Sig. (2-tailed)	.017	
SMB2	Water (control)	14	0.384	Mann-Whitney U	2	
	Shellac	2	0.507	Asymp. Sig. (2-tailed)	.057	
	Total	16		Exact Sig. (2-tailed)	.067	
TYA1	Water (control)	14	1.818	Mann-Whitney U	5	Shellac = unsafe
	Shellac	2	1.557	Asymp. Sig. (2-tailed)	.153	
	Total	16		Exact Sig. (2-tailed)	.200	
TYA2	Water (control)	14	1.848	Mann-Whitney U	0	
	Shellac	2	1.422	Asymp. Sig. (2-tailed)	.026	
	Total	16		Exact Sig. (2-tailed)	.017	
TYB1	Water (control)	14	2.652	Mann-Whitney U	0	
	Shellac	2	1.995	Asymp. Sig. (2-tailed)	.026	
	Total	16		Exact Sig. (2-tailed)	.017	
TYB2	Water (control)	14	2.669	Mann-Whitney U	0	
	Shellac	2	2.109	Asymp. Sig. (2-tailed)	.026	
	Total	16		Exact Sig. (2-tailed)	.017	
TYC1	Water (control)	14	2.108	Mann-Whitney U	0	
	Shellac	2	1.435	Asymp. Sig. (2-tailed)	.026	
	Total	16		Exact Sig. (2-tailed)	.017	
TYC2	Water (control)	14	2.136	Mann-Whitney U	0	
	Shellac	2	1.012	Asymp. Sig. (2-tailed)	.026	
	Total	16		Exact Sig. (2-tailed)	.017	
TYD1	Water (control)	14	1.809	Mann-Whitney U	10	
	Shellac	2	1.498	Asymp. Sig. (2-tailed)	.525	
	Total	16		Exact Sig. (2-tailed)	.600	
TYD2	Water (control)	14	1.832	Mann-Whitney U	0	
	Shellac	2	0.996	Asymp. Sig. (2-tailed)	.026	
	Total	16		Exact Sig. (2-tailed)	.017	
TYE1	Water (control)	14	4.148	Mann-Whitney U	12	
	Shellac	2	3.038	Asymp. Sig. (2-tailed)	.751	
	Total	16		Exact Sig. (2-tailed)	.817	
TYE2	Water (control)	14	4.094	Mann-Whitney U	0	
	Shellac	2	1.535	Asymp. Sig. (2-tailed)	.026	
	Total	16		Exact Sig. (2-tailed)	.017	

Peak	Treatment	N	Median	Test statistics		Conclusion
SMA1	Water (control)	14	0.934	Mann-Whitney U	19	Sodium bicarbonate = safe
	Sodium bicarbonate	3	0.939	Asymp. Sig. (2-tailed)	.801	
	Total	17		Exact Sig. (2-tailed)	.844	
SMB1	Water (control)	14	0.393	Mann-Whitney U	0	
	Sodium bicarbonate	2	1.062	Asymp. Sig. (2-tailed)	.026	
	Total	16		Exact Sig. (2-tailed)	.017	
TYA1	Water (control)	14	1.818	Mann-Whitney U	1	Sodium bicarbonate = unsafe
	Sodium bicarbonate	3	1.097	Asymp. Sig. (2-tailed)	.012	
	Total	17		Exact Sig. (2-tailed)	.006	
TYA2	Water (control)	14	1.848	Mann-Whitney U	0	
	Sodium bicarbonate	3	1.241	Asymp. Sig. (2-tailed)	.008	
	Total	17		Exact Sig. (2-tailed)	.003	
TYB1	Water (control)	14	2.652	Mann-Whitney U	0	
	Sodium bicarbonate	3	1.360	Asymp. Sig. (2-tailed)	.008	
	Total	17		Exact Sig. (2-tailed)	.003	
TYC1	Water (control)	14	2.108	Mann-Whitney U	0	
	Sodium bicarbonate	3	1.000	Asymp. Sig. (2-tailed)	.008	
	Total	17		Exact Sig. (2-tailed)	.003	
TYC2	Water (control)	14	2.136	Mann-Whitney U	0	
	Sodium bicarbonate	3	1.079	Asymp. Sig. (2-tailed)	.008	
	Total	17		Exact Sig. (2-tailed)	.003	
TYD1	Water (control)	14	1.809	Mann-Whitney U	0	
	Sodium bicarbonate	2	1.048	Asymp. Sig. (2-tailed)	.026	
	Total	16		Exact Sig. (2-tailed)	.017	
TYE1	Water (control)	14	4.148	Mann-Whitney U	0	
	Sodium bicarbonate	2	1.171	Asymp. Sig. (2-tailed)	.026	
	Total	16		Exact Sig. (2-tailed)	.017	
TYE2	Water (control)	14	4.094	Mann-Whitney U	0	
	Sodium bicarbonate	2	1.234	Asymp. Sig. (2-tailed)	.026	
	Total	16		Exact Sig. (2-tailed)	.017	
SMA1	Water (control)	14	0.934	Mann-Whitney U	0	Heating sodium bicarbonate to 80°C for 24 hours = unsafe
	Sodium bicarbonate - 24H @ 80°C	3	0.136	Asymp. Sig. (2-tailed)	.008	
	Total	17		Exact Sig. (2-tailed)	.003	
SMB1	Water (control)	14	0.393	Mann-Whitney U	0	
	Sodium bicarbonate - 24H @ 80°C	2	0.059	Asymp. Sig. (2-tailed)	.026	
	Total	16		Exact Sig. (2-tailed)	.017	
SMB2	Water (control)	14	0.384	Mann-Whitney U	0	
	Sodium bicarbonate - 24H @ 80°C	2	0.131	Asymp. Sig. (2-tailed)	.026	
	Total	16		Exact Sig. (2-tailed)	.017	
TYA1	Water (control)	14	1.818	Mann-Whitney U	0	Heating sodium bicarbonate to 80°C for 24 hours = unsafe
	Sodium bicarbonate - 24H @ 80°C	3	0.468	Asymp. Sig. (2-tailed)	.008	
	Total	17		Exact Sig. (2-tailed)	.003	
TYA2	Water (control)	14	1.848	Mann-Whitney U	0	
	Sodium bicarbonate - 24H @ 80°C	3	0.415	Asymp. Sig. (2-tailed)	.008	
	Total	17		Exact Sig. (2-tailed)	.003	
TYB1	Water (control)	14	2.652	Mann-Whitney U	0	
	Sodium bicarbonate - 24H @ 80°C	3	0.642	Asymp. Sig. (2-tailed)	.008	
	Total	17		Exact Sig. (2-tailed)	.003	
TYB2	Water (control)	14	2.669	Mann-Whitney U	0	
	Sodium bicarbonate - 24H @ 80°C	3	0.527	Asymp. Sig. (2-tailed)	.008	
	Total	17		Exact Sig. (2-tailed)	.003	
TYC1	Water (control)	14	2.108	Mann-Whitney U	0	
	Sodium bicarbonate - 24H @ 80°C	3	0.342	Asymp. Sig. (2-tailed)	.008	
	Total	17		Exact Sig. (2-tailed)	.003	
TYC2	Water (control)	14	2.136	Mann-Whitney U	0	
	Sodium bicarbonate - 24H @ 80°C	3	0.302	Asymp. Sig. (2-tailed)	.008	
	Total	17		Exact Sig. (2-tailed)	.003	
TYD1	Water (control)	14	1.809	Mann-Whitney U	0	
	Sodium bicarbonate - 24H @ 80°C	3	0.286	Asymp. Sig. (2-tailed)	.008	
	Total	17		Exact Sig. (2-tailed)	.003	
TYD2	Water (control)	14	1.832	Mann-Whitney U	0	

Peak	Treatment	N	Median	Test statistics		Conclusion
	Sodium bicarbonate - 24H @ 80°C	3	0.250	Asymp. Sig. (2-tailed)	.008	
	Total	17		Exact Sig. (2-tailed)	.003	
TYE1	Water (control)	14	4.148	Mann-Whitney U	1	
	Sodium bicarbonate - 24H @ 80°C	3	0.419	Asymp. Sig. (2-tailed)	.012	
	Total	17		Exact Sig. (2-tailed)	.006	
TYE2	Water (control)	14	4.094	Mann-Whitney U	0	
	Sodium bicarbonate - 24H @ 80°C	3	0.375	Asymp. Sig. (2-tailed)	.008	
	Total	17		Exact Sig. (2-tailed)	.003	
SMA1	Water (control)	14	0.934	Mann-Whitney U	4	
	Sodium carbonate	4	1.138	Asymp. Sig. (2-tailed)	.011	
	Total	18		Exact Sig. (2-tailed)	.007	
SMA2	Water (control)	14	0.897	Mann-Whitney U	2	
	Sodium carbonate	4	1.129	Asymp. Sig. (2-tailed)	.006	
	Total	18		Exact Sig. (2-tailed)	.003	Sodium carbonate = safe
SMB1	Water (control)	14	0.393	Mann-Whitney U	1	
	Sodium carbonate	4	0.609	Asymp. Sig. (2-tailed)	.004	
	Total	18		Exact Sig. (2-tailed)	.001	
SMB2	Water (control)	14	0.384	Mann-Whitney U	3	
	Sodium carbonate	4	0.641	Asymp. Sig. (2-tailed)	.008	
	Total	18		Exact Sig. (2-tailed)	.005	
TYA1	Water (control)	14	1.818	Mann-Whitney U	0	
	Sodium carbonate	4	1.342	Asymp. Sig. (2-tailed)	.003	
	Total	18		Exact Sig. (2-tailed)	.001	
TYA2	Water (control)	14	1.848	Mann-Whitney U	0	
	Sodium carbonate	4	1.370	Asymp. Sig. (2-tailed)	.003	
	Total	18		Exact Sig. (2-tailed)	.001	
TYB1	Water (control)	14	2.652	Mann-Whitney U	0	
	Sodium carbonate	4	1.732	Asymp. Sig. (2-tailed)	.003	
	Total	18		Exact Sig. (2-tailed)	.001	
TYB2	Water (control)	14	2.669	Mann-Whitney U	0	
	Sodium carbonate	4	1.766	Asymp. Sig. (2-tailed)	.003	
	Total	18		Exact Sig. (2-tailed)	.001	
TYC1	Water (control)	14	2.108	Mann-Whitney U	21	
	Sodium carbonate	4	1.583	Asymp. Sig. (2-tailed)	.457	
	Total	18		Exact Sig. (2-tailed)	.505	Sodium carbonate = unsafe
TYC2	Water (control)	14	2.136	Mann-Whitney U	2	
	Sodium carbonate	4	0.959	Asymp. Sig. (2-tailed)	.006	
	Total	18		Exact Sig. (2-tailed)	.003	
TYD1	Water (control)	14	1.809	Mann-Whitney U	0	
	Sodium carbonate	4	0.934	Asymp. Sig. (2-tailed)	.003	
	Total	18		Exact Sig. (2-tailed)	.001	
TYD2	Water (control)	14	1.832	Mann-Whitney U	0	
	Sodium carbonate	4	0.822	Asymp. Sig. (2-tailed)	.003	
	Total	18		Exact Sig. (2-tailed)	.001	
TYE1	Water (control)	14	4.148	Mann-Whitney U	0	
	Sodium carbonate	4	1.619	Asymp. Sig. (2-tailed)	.003	
	Total	18		Exact Sig. (2-tailed)	.001	
TYE2	Water (control)	14	4.094	Mann-Whitney U	0	
	Sodium carbonate	4	1.242	Asymp. Sig. (2-tailed)	.003	
	Total	18		Exact Sig. (2-tailed)	.001	
SMA1	Water (control)	14	0.934	Mann-Whitney U	18	
	Sodium chloride	4	1.078	Asymp. Sig. (2-tailed)	.288	
	Total	18		Exact Sig. (2-tailed)	.318	
SMA2	Water (control)	14	0.897	Mann-Whitney U	5	
	Sodium chloride	4	1.030	Asymp. Sig. (2-tailed)	.015	
	Total	18		Exact Sig. (2-tailed)	.012	Sodium chloride = safe
SMB1	Water (control)	14	0.393	Mann-Whitney U	16	
	Sodium chloride	4	0.489	Asymp. Sig. (2-tailed)	.203	
	Total	18		Exact Sig. (2-tailed)	.233	
SMB2	Water (control)	14	0.384	Mann-Whitney U	8	
	Sodium chloride	4	0.455	Asymp. Sig. (2-tailed)	.034	
	Total	18		Exact Sig. (2-tailed)	.035	

Peak	Treatment	N	Median	Test statistics		Conclusion
TYA1	Water (control)	14	1.818	Mann-Whitney U	23	Sodium chloride = safe
	Sodium chloride	4	1.787	Asymp. Sig. (2-tailed)	.595	
	Total	18		Exact Sig. (2-tailed)	.645	
TYA2	Water (control)	14	1.848	Mann-Whitney U	28	
	Sodium chloride	4	1.891	Asymp. Sig. (2-tailed)	1.000	
	Total	18		Exact Sig. (2-tailed)	1.000	
TYB1	Water (control)	14	2.652	Mann-Whitney U	23	
	Sodium chloride	4	2.609	Asymp. Sig. (2-tailed)	.595	
	Total	18		Exact Sig. (2-tailed)	.645	
TYB2	Water (control)	14	2.669	Mann-Whitney U	28	
	Sodium chloride	4	2.748	Asymp. Sig. (2-tailed)	1.000	
	Total	18		Exact Sig. (2-tailed)	1.000	
TYC1	Water (control)	14	2.108	Mann-Whitney U	27	
	Sodium chloride	4	2.101	Asymp. Sig. (2-tailed)	.915	
	Total	18		Exact Sig. (2-tailed)	.959	
TYC2	Water (control)	14	2.136	Mann-Whitney U	25	
	Sodium chloride	4	2.312	Asymp. Sig. (2-tailed)	.750	
	Total	18		Exact Sig. (2-tailed)	.798	
TYD1	Water (control)	14	1.809	Mann-Whitney U	19.5	
	Sodium chloride	4	1.930	Asymp. Sig. (2-tailed)	.366	
	Total	18		Exact Sig. (2-tailed)	.392	
TYD2	Water (control)	14	1.832	Mann-Whitney U	10	
	Sodium chloride	4	2.098	Asymp. Sig. (2-tailed)	.056	
	Total	18		Exact Sig. (2-tailed)	.061	
TYE1	Water (control)	14	4.148	Mann-Whitney U	27.5	
	Sodium chloride	4	4.090	Asymp. Sig. (2-tailed)	.958	
	Total	18		Exact Sig. (2-tailed)	.975	
TYE2	Water (control)	14	4.094	Mann-Whitney U	12	
	Sodium chloride	4	4.290	Asymp. Sig. (2-tailed)	.089	
	Total	18		Exact Sig. (2-tailed)	.101	
SMA1	Water (control)	14	0.934	Mann-Whitney U	30	Heating sodium chloride to 37°C for 24 hours = safe
	Sodium chloride - 24H @ 37°C	5	1.022	Asymp. Sig. (2-tailed)	.643	
	Total	19		Exact Sig. (2-tailed)	.673	
SMA2	Water (control)	14	0.897	Mann-Whitney U	20	
	Sodium chloride - 24H @ 37°C	5	1.012	Asymp. Sig. (2-tailed)	.165	
	Total	19		Exact Sig. (2-tailed)	.186	
SMB1	Water (control)	14	0.393	Mann-Whitney U	19	
	Sodium chloride - 24H @ 37°C	5	0.461	Asymp. Sig. (2-tailed)	.139	
	Total	19		Exact Sig. (2-tailed)	.156	
SMB2	Water (control)	14	0.384	Mann-Whitney U	2	
	Sodium chloride - 24H @ 37°C	5	0.538	Asymp. Sig. (2-tailed)	.002	
	Total	19		Exact Sig. (2-tailed)	.001	
TYA1	Water (control)	14	1.818	Mann-Whitney U	32	Heating sodium chloride to 37°C for 24 hours = mixed result
	Sodium chloride - 24H @ 37°C	5	1.821	Asymp. Sig. (2-tailed)	.781	
	Total	19		Exact Sig. (2-tailed)	.823	
TYA2	Water (control)	14	1.848	Mann-Whitney U	31	
	Sodium chloride - 24H @ 37°C	5	2.031	Asymp. Sig. (2-tailed)	.711	
	Total	19		Exact Sig. (2-tailed)	.754	
TYB1	Water (control)	14	2.652	Mann-Whitney U	32	
	Sodium chloride - 24H @ 37°C	5	2.749	Asymp. Sig. (2-tailed)	.781	
	Total	19		Exact Sig. (2-tailed)	.823	
TYB2	Water (control)	14	2.669	Mann-Whitney U	31	
	Sodium chloride - 24H @ 37°C	5	2.884	Asymp. Sig. (2-tailed)	.711	
	Total	19		Exact Sig. (2-tailed)	.754	
TYC1	Water (control)	14	2.108	Mann-Whitney U	28	
	Sodium chloride - 24H @ 37°C	5	2.301	Asymp. Sig. (2-tailed)	.517	
	Total	19		Exact Sig. (2-tailed)	.559	
TYC2	Water (control)	14	2.136	Mann-Whitney U	0	
	Sodium chloride - 24H @ 37°C	5	1.388	Asymp. Sig. (2-tailed)	.001	
	Total	19		Exact Sig. (2-tailed)	.000	
TYD1	Water (control)	14	1.809	Mann-Whitney U	6	
	Sodium chloride - 24H @ 37°C	5	2.066	Asymp. Sig. (2-tailed)	.007	

Peak	Treatment	N	Median	Test statistics		Conclusion
	Total	19		Exact Sig. (2-tailed)	.005	
TYD2	Water (control)	14	1.832	Mann-Whitney U	0	
	Sodium chloride - 24H @ 37°C	5	1.294	Asymp. Sig. (2-tailed)	.001	
	Total	19		Exact Sig. (2-tailed)	.000	
TYE1	Water (control)	14	4.148	Mann-Whitney U	33	
	Sodium chloride - 24H @ 37°C	5	4.026	Asymp. Sig. (2-tailed)	.853	
	Total	19		Exact Sig. (2-tailed)	.893	
TYE2	Water (control)	14	4.094	Mann-Whitney U	0	
	Sodium chloride - 24H @ 37°C	5	1.880	Asymp. Sig. (2-tailed)	.001	
	Total	19		Exact Sig. (2-tailed)	.000	
SMA1	Water (control)	14	0.934	Mann-Whitney U	0	Sodium hydroxide = unsafe
	Sodium hydroxide	4	0.610	Asymp. Sig. (2-tailed)	.003	
	Total	18		Exact Sig. (2-tailed)	.001	
SMA2	Water (control)	14	0.897	Mann-Whitney U	0	
	Sodium hydroxide	4	0.614	Asymp. Sig. (2-tailed)	.003	
	Total	18		Exact Sig. (2-tailed)	.001	
SMB1	Water (control)	14	0.393	Mann-Whitney U	0	
	Sodium hydroxide	4	0.252	Asymp. Sig. (2-tailed)	.003	
	Total	18		Exact Sig. (2-tailed)	.001	
SMB2	Water (control)	14	0.384	Mann-Whitney U	0	
	Sodium hydroxide	4	0.248	Asymp. Sig. (2-tailed)	.003	
	Total	18		Exact Sig. (2-tailed)	.001	
TYA1	Water (control)	14	1.818	Mann-Whitney U	1	Sodium hydroxide = unsafe
	Sodium hydroxide	4	1.368	Asymp. Sig. (2-tailed)	.004	
	Total	18		Exact Sig. (2-tailed)	.001	
TYA2	Water (control)	14	1.848	Mann-Whitney U	0	
	Sodium hydroxide	4	1.392	Asymp. Sig. (2-tailed)	.003	
	Total	18		Exact Sig. (2-tailed)	.001	
TYB1	Water (control)	14	2.652	Mann-Whitney U	0	
	Sodium hydroxide	4	2.017	Asymp. Sig. (2-tailed)	.003	
	Total	18		Exact Sig. (2-tailed)	.001	
TYB2	Water (control)	14	2.669	Mann-Whitney U	0	
	Sodium hydroxide	4	2.034	Asymp. Sig. (2-tailed)	.003	
	Total	18		Exact Sig. (2-tailed)	.001	
TYC1	Water (control)	14	2.108	Mann-Whitney U	0	
	Sodium hydroxide	4	1.721	Asymp. Sig. (2-tailed)	.003	
	Total	18		Exact Sig. (2-tailed)	.001	
TYC2	Water (control)	14	2.136	Mann-Whitney U	0	
	Sodium hydroxide	4	1.758	Asymp. Sig. (2-tailed)	.003	
	Total	18		Exact Sig. (2-tailed)	.001	
TYD1	Water (control)	14	1.809	Mann-Whitney U	17	
	Sodium hydroxide	4	1.711	Asymp. Sig. (2-tailed)	.243	
	Total	18		Exact Sig. (2-tailed)	.277	
TYD2	Water (control)	14	1.832	Mann-Whitney U	14	
	Sodium hydroxide	4	1.738	Asymp. Sig. (2-tailed)	.137	
	Total	18		Exact Sig. (2-tailed)	.158	
TYE1	Water (control)	14	4.148	Mann-Whitney U	19	
	Sodium hydroxide	4	3.782	Asymp. Sig. (2-tailed)	.339	
	Total	18		Exact Sig. (2-tailed)	.382	
TYE2	Water (control)	14	4.094	Mann-Whitney U	19	
	Sodium hydroxide	4	3.891	Asymp. Sig. (2-tailed)	.339	
	Total	18		Exact Sig. (2-tailed)	.382	
SMA1	Water (control)	14	0.934	Mann-Whitney U	0	Sodium sulphide = unsafe
	Sodium sulfide	4	0.095	Asymp. Sig. (2-tailed)	.003	
	Total	18		Exact Sig. (2-tailed)	.001	
SMA2	Water (control)	14	0.897	Mann-Whitney U	0	
	Sodium sulfide	4	0.086	Asymp. Sig. (2-tailed)	.003	
	Total	18		Exact Sig. (2-tailed)	.001	
SMB1	Water (control)	14	0.393	Mann-Whitney U	0	
	Sodium sulfide	4	0.068	Asymp. Sig. (2-tailed)	.003	
	Total	18		Exact Sig. (2-tailed)	.001	
SMB2	Water (control)	14	0.384	Mann-Whitney U	0	

Peak	Treatment	N	Median	Test statistics		Conclusion
	Sodium sulfide	2	0.050	Asymp. Sig. (2-tailed)	.026	
	Total	16		Exact Sig. (2-tailed)	.017	
TYA1	Water (control)	14	1.818	Mann-Whitney U	0	Sodium sulphide = unsafe
	Sodium sulfide	4	0.168	Asymp. Sig. (2-tailed)	.003	
	Total	18		Exact Sig. (2-tailed)	.001	
TYA2	Water (control)	14	1.848	Mann-Whitney U	0	
	Sodium sulfide	4	0.192	Asymp. Sig. (2-tailed)	.003	
	Total	18		Exact Sig. (2-tailed)	.001	
TYB1	Water (control)	14	2.652	Mann-Whitney U	0	
	Sodium sulfide	3	0.278	Asymp. Sig. (2-tailed)	.008	
	Total	17		Exact Sig. (2-tailed)	.003	
TYB2	Water (control)	14	2.669	Mann-Whitney U	0	
	Sodium sulfide	4	0.253	Asymp. Sig. (2-tailed)	.003	
	Total	18		Exact Sig. (2-tailed)	.001	
TYC1	Water (control)	14	2.108	Mann-Whitney U	0	
	Sodium sulfide	3	0.127	Asymp. Sig. (2-tailed)	.008	
	Total	17		Exact Sig. (2-tailed)	.003	
TYC2	Water (control)	14	2.136	Mann-Whitney U	0	
	Sodium sulfide	4	0.122	Asymp. Sig. (2-tailed)	.003	
	Total	18		Exact Sig. (2-tailed)	.001	
TYD1	Water (control)	14	1.809	Mann-Whitney U	0	
	Sodium sulfide	3	0.133	Asymp. Sig. (2-tailed)	.008	
	Total	17		Exact Sig. (2-tailed)	.003	
TYD2	Water (control)	14	1.832	Mann-Whitney U	0	
	Sodium sulfide	4	0.122	Asymp. Sig. (2-tailed)	.003	
	Total	18		Exact Sig. (2-tailed)	.001	
TYE1	Water (control)	14	4.148	Mann-Whitney U	0	
	Sodium sulfide	3	0.209	Asymp. Sig. (2-tailed)	.008	
	Total	17		Exact Sig. (2-tailed)	.003	
TYE2	Water (control)	14	4.094	Mann-Whitney U	0	
	Sodium sulfide	4	0.185	Asymp. Sig. (2-tailed)	.003	
	Total	18		Exact Sig. (2-tailed)	.001	
SMA1	Water (control)	14	0.934	Mann-Whitney U	0	Heating sodium sulfide to 80°C for 6 hours = unsafe
	Sodium sulfide - 6H @ 80°C	3	0.090	Asymp. Sig. (2-tailed)	.008	
	Total	17		Exact Sig. (2-tailed)	.003	
SMA2	Water (control)	14	0.897	Mann-Whitney U	0	
	Sodium sulfide - 6H @ 80°C	3	0.093	Asymp. Sig. (2-tailed)	.008	
	Total	17		Exact Sig. (2-tailed)	.003	
SMB1	Water (control)	14	0.393	Mann-Whitney U	0	
	Sodium sulfide - 6H @ 80°C	3	0.057	Asymp. Sig. (2-tailed)	.008	
	Total	17		Exact Sig. (2-tailed)	.003	
SMB2	Water (control)	14	0.384	Mann-Whitney U	0	
	Sodium sulfide - 6H @ 80°C	2	0.066	Asymp. Sig. (2-tailed)	.026	
	Total	16		Exact Sig. (2-tailed)	.017	
TYA1	Water (control)	14	1.818	Mann-Whitney U	0	Heating sodium sulfide to 80°C for 6 hours = unsafe
	Sodium sulfide - 6H @ 80°C	4	0.370	Asymp. Sig. (2-tailed)	.003	
	Total	18		Exact Sig. (2-tailed)	.001	
TYA2	Water (control)	14	1.848	Mann-Whitney U	0	
	Sodium sulfide - 6H @ 80°C	3	0.362	Asymp. Sig. (2-tailed)	.008	
	Total	17		Exact Sig. (2-tailed)	.003	
TYB1	Water (control)	14	2.652	Mann-Whitney U	0	
	Sodium sulfide - 6H @ 80°C	3	0.522	Asymp. Sig. (2-tailed)	.008	
	Total	17		Exact Sig. (2-tailed)	.003	
TYB2	Water (control)	14	2.669	Mann-Whitney U	0	
	Sodium sulfide - 6H @ 80°C	3	0.503	Asymp. Sig. (2-tailed)	.008	
	Total	17		Exact Sig. (2-tailed)	.003	
TYC1	Water (control)	14	2.108	Mann-Whitney U	0	
	Sodium sulfide - 6H @ 80°C	3	0.254	Asymp. Sig. (2-tailed)	.008	
	Total	17		Exact Sig. (2-tailed)	.003	
TYC2	Water (control)	14	2.136	Mann-Whitney U	0	
	Sodium sulfide - 6H @ 80°C	3	0.253	Asymp. Sig. (2-tailed)	.008	
	Total	17		Exact Sig. (2-tailed)	.003	

Peak	Treatment	N	Median	Test statistics		Conclusion
TYD1	Water (control)	14	1.809	Mann-Whitney U	0	
	Sodium sulfide - 6H @ 80°C	3	0.245	Asymp. Sig. (2-tailed)	.008	
	Total	17		Exact Sig. (2-tailed)	.003	
TYD2	Water (control)	14	1.832	Mann-Whitney U	0	
	Sodium sulfide - 6H @ 80°C	3	0.235	Asymp. Sig. (2-tailed)	.008	
	Total	17		Exact Sig. (2-tailed)	.003	
TYE1	Water (control)	14	4.148	Mann-Whitney U	0	
	Sodium sulfide - 6H @ 80°C	3	0.369	Asymp. Sig. (2-tailed)	.008	
	Total	17		Exact Sig. (2-tailed)	.003	
TYE2	Water (control)	14	4.094	Mann-Whitney U	0	
	Sodium sulfide - 6H @ 80°C	3	0.359	Asymp. Sig. (2-tailed)	.008	
	Total	17		Exact Sig. (2-tailed)	.003	
SMA1	Water (control)	14	0.934	Mann-Whitney U	26	
	Toluene	4	0.936	Asymp. Sig. (2-tailed)	.832	
	Total	18		Exact Sig. (2-tailed)	.862	
SMA2	Water (control)	14	0.897	Mann-Whitney U	26	
	Toluene	4	0.900	Asymp. Sig. (2-tailed)	.832	
	Total	18		Exact Sig. (2-tailed)	.878	Toluene = safe
SMB1	Water (control)	14	0.393	Mann-Whitney U	23	
	Toluene	4	0.414	Asymp. Sig. (2-tailed)	.595	
	Total	18		Exact Sig. (2-tailed)	.645	
SMB2	Water (control)	14	0.384	Mann-Whitney U	22.5	
	Toluene	4	0.402	Asymp. Sig. (2-tailed)	.559	
	Total	18		Exact Sig. (2-tailed)	.587	
TYA1	Water (control)	14	1.818	Mann-Whitney U	26.5	
	Toluene	4	1.801	Asymp. Sig. (2-tailed)	.873	
	Total	18		Exact Sig. (2-tailed)	.894	
TYA2	Water (control)	14	1.848	Mann-Whitney U	22	
	Toluene	4	1.806	Asymp. Sig. (2-tailed)	.524	
	Total	18		Exact Sig. (2-tailed)	.574	
TYB1	Water (control)	14	2.652	Mann-Whitney U	27	
	Toluene	4	2.614	Asymp. Sig. (2-tailed)	.915	
	Total	18		Exact Sig. (2-tailed)	.959	
TYB2	Water (control)	14	2.669	Mann-Whitney U	23	
	Toluene	4	2.606	Asymp. Sig. (2-tailed)	.595	
	Total	18		Exact Sig. (2-tailed)	.645	
TYC1	Water (control)	14	2.108	Mann-Whitney U	15	
	Toluene	4	2.265	Asymp. Sig. (2-tailed)	.167	
	Total	18		Exact Sig. (2-tailed)	.192	
TYC2	Water (control)	14	2.136	Mann-Whitney U	21	Toluene = safe
	Toluene	4	2.253	Asymp. Sig. (2-tailed)	.457	
	Total	18		Exact Sig. (2-tailed)	.505	
TYD1	Water (control)	14	1.809	Mann-Whitney U	15	
	Toluene	4	2.020	Asymp. Sig. (2-tailed)	.167	
	Total	18		Exact Sig. (2-tailed)	.192	
TYD2	Water (control)	14	1.832	Mann-Whitney U	21	
	Toluene	4	2.037	Asymp. Sig. (2-tailed)	.457	
	Total	18		Exact Sig. (2-tailed)	.505	
TYE1	Water (control)	14	4.148	Mann-Whitney U	25	
	Toluene	4	4.259	Asymp. Sig. (2-tailed)	.750	
	Total	18		Exact Sig. (2-tailed)	.798	
TYE2	Water (control)	14	4.094	Mann-Whitney U	23	
	Toluene	4	4.374	Asymp. Sig. (2-tailed)	.595	
	Total	18		Exact Sig. (2-tailed)	.645	
SMA1	Water (control)	14	0.934	Mann-Whitney U	23	Trichloro-
	Trichloroethylene	4	0.913	Asymp. Sig. (2-tailed)	.595	ethylene = safe
	Total	18		Exact Sig. (2-tailed)	.631	
SMA2	Water (control)	14	0.897	Mann-Whitney U	22.5	
	Trichloroethylene	4	0.913	Asymp. Sig. (2-tailed)	.559	
	Total	18		Exact Sig. (2-tailed)	.588	
SMB1	Water (control)	14	0.393	Mann-Whitney U	12	
	Trichloroethylene	4	0.414	Asymp. Sig. (2-tailed)	.089	

Peak	Treatment	N	Median	Test statistics		Conclusion
	Total	18		Exact Sig. (2-tailed)	.101	
SMB2	Water (control)	14	0.384	Mann-Whitney U	16.5	
	Trichloroethylene	4	0.406	Asymp. Sig. (2-tailed)	.222	
	Total	18		Exact Sig. (2-tailed)	.241	
TYA1	Water (control)	14	1.818	Mann-Whitney U	21	Trichloro-ethylene = safe
	Trichloroethylene	4	1.844	Asymp. Sig. (2-tailed)	.457	
	Total	18		Exact Sig. (2-tailed)	.505	
TYA2	Water (control)	14	1.848	Mann-Whitney U	27.5	
	Trichloroethylene	4	1.826	Asymp. Sig. (2-tailed)	.958	
	Total	18		Exact Sig. (2-tailed)	.975	
TYB1	Water (control)	14	2.652	Mann-Whitney U	25	
	Trichloroethylene	4	2.668	Asymp. Sig. (2-tailed)	.750	
	Total	18		Exact Sig. (2-tailed)	.798	
TYB2	Water (control)	14	2.669	Mann-Whitney U	28	
	Trichloroethylene	4	2.629	Asymp. Sig. (2-tailed)	1.000	
	Total	18		Exact Sig. (2-tailed)	1.000	
TYC1	Water (control)	14	2.108	Mann-Whitney U	18	
	Trichloroethylene	4	2.275	Asymp. Sig. (2-tailed)	.288	
	Total	18		Exact Sig. (2-tailed)	.327	
TYC2	Water (control)	14	2.136	Mann-Whitney U	19	
	Trichloroethylene	4	2.322	Asymp. Sig. (2-tailed)	.339	
	Total	18		Exact Sig. (2-tailed)	.382	
TYD1	Water (control)	14	1.809	Mann-Whitney U	8	
	Trichloroethylene	4	2.061	Asymp. Sig. (2-tailed)	.034	
	Total	18		Exact Sig. (2-tailed)	.035	
TYD2	Water (control)	14	1.832	Mann-Whitney U	11	
	Trichloroethylene	4	2.042	Asymp. Sig. (2-tailed)	.071	
	Total	18		Exact Sig. (2-tailed)	.079	
TYE1	Water (control)	14	4.148	Mann-Whitney U	22	
	Trichloroethylene	4	4.243	Asymp. Sig. (2-tailed)	.524	
	Total	18		Exact Sig. (2-tailed)	.574	
TYE2	Water (control)	14	4.094	Mann-Whitney U	21	
	Trichloroethylene	4	4.239	Asymp. Sig. (2-tailed)	.457	
	Total	18		Exact Sig. (2-tailed)	.505	
TYA1	Water (control)	14	1.818	Mann-Whitney U	0	Turpentine = unsafe
	Turpentine	4	0.217	Asymp. Sig. (2-tailed)	.003	
	Total	18		Exact Sig. (2-tailed)	.001	
TYA2	Water (control)	14	1.848	Mann-Whitney U	0	
	Turpentine	4	0.220	Asymp. Sig. (2-tailed)	.003	
	Total	18		Exact Sig. (2-tailed)	.001	
TYB1	Water (control)	14	2.652	Mann-Whitney U	0	
	Turpentine	4	0.321	Asymp. Sig. (2-tailed)	.003	
	Total	18		Exact Sig. (2-tailed)	.001	
TYB2	Water (control)	14	2.669	Mann-Whitney U	0	
	Turpentine	4	0.319	Asymp. Sig. (2-tailed)	.003	
	Total	18		Exact Sig. (2-tailed)	.001	
TYC1	Water (control)	14	2.108	Mann-Whitney U	0	
	Turpentine	4	0.149	Asymp. Sig. (2-tailed)	.003	
	Total	18		Exact Sig. (2-tailed)	.001	
TYC2	Water (control)	14	2.136	Mann-Whitney U	0	
	Turpentine	4	0.131	Asymp. Sig. (2-tailed)	.003	
	Total	18		Exact Sig. (2-tailed)	.001	
TYD1	Water (control)	14	1.809	Mann-Whitney U	0	
	Turpentine	4	0.138	Asymp. Sig. (2-tailed)	.003	
	Total	18		Exact Sig. (2-tailed)	.001	
TYD2	Water (control)	14	1.832	Mann-Whitney U	0	
	Turpentine	4	0.125	Asymp. Sig. (2-tailed)	.003	
	Total	18		Exact Sig. (2-tailed)	.001	
TYE1	Water (control)	14	4.148	Mann-Whitney U	0	
	Turpentine	4	0.241	Asymp. Sig. (2-tailed)	.003	
	Total	18		Exact Sig. (2-tailed)	.001	
TYE2	Water (control)	14	4.094	Mann-Whitney U	0	

Peak	Treatment	N	Median	Test statistics		Conclusion
	Turpentine	4	0.209	Asymp. Sig. (2-tailed)	.003	
	Total	18		Exact Sig. (2-tailed)	.001	
SMA1	Water (control)	14	0.934	Mann-Whitney U	1	
	White spirit	4	0.766	Asymp. Sig. (2-tailed)	.004	
	Total	18		Exact Sig. (2-tailed)	.001	
SMA2	Water (control)	14	0.897	Mann-Whitney U	2	
	White spirit	4	0.737	Asymp. Sig. (2-tailed)	.006	
	Total	18		Exact Sig. (2-tailed)	.003	White spirit =
SMB1	Water (control)	14	0.393	Mann-Whitney U	11	unsafe
	White spirit	4	0.329	Asymp. Sig. (2-tailed)	.071	
	Total	18		Exact Sig. (2-tailed)	.079	
SMB2	Water (control)	14	0.384	Mann-Whitney U	2	
	White spirit	4	0.311	Asymp. Sig. (2-tailed)	.006	
	Total	18		Exact Sig. (2-tailed)	.003	
TYA1	Water (control)	14	1.818	Mann-Whitney U	11	
	White spirit	4	1.601	Asymp. Sig. (2-tailed)	.071	
	Total	18		Exact Sig. (2-tailed)	.079	
TYA2	Water (control)	14	1.848	Mann-Whitney U	8	
	White spirit	4	1.611	Asymp. Sig. (2-tailed)	.034	
	Total	18		Exact Sig. (2-tailed)	.035	
TYB1	Water (control)	14	2.652	Mann-Whitney U	7	
	White spirit	4	2.301	Asymp. Sig. (2-tailed)	.026	
	Total	18		Exact Sig. (2-tailed)	.025	
TYB2	Water (control)	14	2.669	Mann-Whitney U	11	
	White spirit	4	2.428	Asymp. Sig. (2-tailed)	.071	
	Total	18		Exact Sig. (2-tailed)	.079	
TYC1	Water (control)	14	2.108	Mann-Whitney U	10	
	White spirit	4	1.916	Asymp. Sig. (2-tailed)	.056	
	Total	18		Exact Sig. (2-tailed)	.061	White spirit =
TYC2	Water (control)	14	2.136	Mann-Whitney U	22	unsafe
	White spirit	4	2.137	Asymp. Sig. (2-tailed)	.524	
	Total	18		Exact Sig. (2-tailed)	.574	
TYD1	Water (control)	14	1.809	Mann-Whitney U	11	
	White spirit	4	1.572	Asymp. Sig. (2-tailed)	.071	
	Total	18		Exact Sig. (2-tailed)	.079	
TYD2	Water (control)	14	1.832	Mann-Whitney U	25	
	White spirit	4	1.946	Asymp. Sig. (2-tailed)	.750	
	Total	18		Exact Sig. (2-tailed)	.798	
TYE1	Water (control)	14	4.148	Mann-Whitney U	12	
	White spirit	4	3.335	Asymp. Sig. (2-tailed)	.089	
	Total	18		Exact Sig. (2-tailed)	.101	
TYE2	Water (control)	14	4.094	Mann-Whitney U	27	
	White spirit	4	4.050	Asymp. Sig. (2-tailed)	.915	
	Total	18		Exact Sig. (2-tailed)	.959	
SMA1	Water (control)	14	0.934	Mann-Whitney U	24	
	Xylene	4	0.928	Asymp. Sig. (2-tailed)	.671	
	Total	18		Exact Sig. (2-tailed)	.706	
SMA2	Water (control)	14	0.897	Mann-Whitney U	17.5	
	Xylene	4	0.868	Asymp. Sig. (2-tailed)	.265	
	Total	18		Exact Sig. (2-tailed)	.285	Xylene = safe
SMB1	Water (control)	14	0.393	Mann-Whitney U	21.5	
	Xylene	4	0.408	Asymp. Sig. (2-tailed)	.490	
	Total	18		Exact Sig. (2-tailed)	.518	
SMB2	Water (control)	14	0.384	Mann-Whitney U	26.5	
	Xylene	4	0.388	Asymp. Sig. (2-tailed)	.873	
	Total	18		Exact Sig. (2-tailed)	.894	
TYA1	Water (control)	14	1.818	Mann-Whitney U	28	Xylene = safe
	Xylene	4	1.759	Asymp. Sig. (2-tailed)	1.000	
	Total	18		Exact Sig. (2-tailed)	1.000	
TYA2	Water (control)	14	1.848	Mann-Whitney U	26	
	Xylene	4	1.844	Asymp. Sig. (2-tailed)	.832	
	Total	18		Exact Sig. (2-tailed)	.878	

Peak	Treatment	N	Median	Test statistics		Conclusion
TYB1	Water (control)	14	2.652	Mann-Whitney U	26	
	Xylene	4	2.542	Asymp. Sig. (2-tailed)	.832	
	Total	18		Exact Sig. (2-tailed)	.878	
TYB2	Water (control)	14	2.669	Mann-Whitney U	26	
	Xylene	4	2.658	Asymp. Sig. (2-tailed)	.832	
	Total	18		Exact Sig. (2-tailed)	.878	
TYC1	Water (control)	14	2.108	Mann-Whitney U	28	
	Xylene	4	2.095	Asymp. Sig. (2-tailed)	1.000	
	Total	18		Exact Sig. (2-tailed)	1.000	
TYC2	Water (control)	14	2.136	Mann-Whitney U	24	
	Xylene	4	2.170	Asymp. Sig. (2-tailed)	.671	
	Total	18		Exact Sig. (2-tailed)	.721	
TYD1	Water (control)	14	1.809	Mann-Whitney U	9	
	Xylene	4	2.056	Asymp. Sig. (2-tailed)	.044	
	Total	18		Exact Sig. (2-tailed)	.046	
TYD2	Water (control)	14	1.832	Mann-Whitney U	16	
	Xylene	4	2.007	Asymp. Sig. (2-tailed)	.203	
	Total	18		Exact Sig. (2-tailed)	.233	
TYE1	Water (control)	14	4.148	Mann-Whitney U	25	
	Xylene	4	4.267	Asymp. Sig. (2-tailed)	.750	
	Total	18		Exact Sig. (2-tailed)	.798	
TYE2	Water (control)	14	4.094	Mann-Whitney U	19	
	Xylene	4	4.311	Asymp. Sig. (2-tailed)	.339	
	Total	18		Exact Sig. (2-tailed)	.382	
SMA2	Water (control)	14	0.897	Mann-Whitney U	13	Gum arabic (re-ppt) = safe
	Gum arabic (re-ppt)	2	0.914	Asymp. Sig. (2-tailed)	.874	
	Total	16		Exact Sig. (2-tailed)	.933	
TYA1	Water (control)	14	1.818	Mann-Whitney U	3	Gum arabic (re-ppt) = unsafe
	Gum arabic (re-ppt)	2	1.475	Asymp. Sig. (2-tailed)	.081	
	Total	16		Exact Sig. (2-tailed)	.100	
TYA2	Water (control)	14	1.848	Mann-Whitney U	0	
	Gum arabic (re-ppt)	2	1.347	Asymp. Sig. (2-tailed)	.026	
	Total	16		Exact Sig. (2-tailed)	.017	
TYB2	Water (control)	14	2.669	Mann-Whitney U	0	
	Gum arabic (re-ppt)	2	1.470	Asymp. Sig. (2-tailed)	.026	
	Total	16		Exact Sig. (2-tailed)	.017	
TYC1	Water (control)	14	2.108	Mann-Whitney U	0	
	Gum arabic (re-ppt)	2	1.026	Asymp. Sig. (2-tailed)	.026	
	Total	16		Exact Sig. (2-tailed)	.017	
TYC2	Water (control)	14	2.136	Mann-Whitney U	0	
	Gum arabic (re-ppt)	2	0.986	Asymp. Sig. (2-tailed)	.026	
	Total	16		Exact Sig. (2-tailed)	.017	
TYD1	Water (control)	14	1.809	Mann-Whitney U	0	
	Gum arabic (re-ppt)	2	0.956	Asymp. Sig. (2-tailed)	.026	
	Total	16		Exact Sig. (2-tailed)	.017	
TYE1	Water (control)	14	4.148	Mann-Whitney U	0	
	Gum arabic (re-ppt)	2	1.178	Asymp. Sig. (2-tailed)	.026	
	Total	16		Exact Sig. (2-tailed)	.017	
TYE2	Water (control)	14	4.094	Mann-Whitney U	0	
	Gum arabic (re-ppt)	2	1.245	Asymp. Sig. (2-tailed)	.026	
	Total	16		Exact Sig. (2-tailed)	.017	
SMA1	Water (control)	14	0.934	Mann-Whitney U	3	Sodium bicarbonate (re-ppt) = safe
	Sodium bicarbonate (re-ppt)	2	0.799	Asymp. Sig. (2-tailed)	.080	
	Total	16		Exact Sig. (2-tailed)	.100	
SMA2	Water (control)	14	0.897	Mann-Whitney U	13	
	Sodium bicarbonate (re-ppt)	2	0.997	Asymp. Sig. (2-tailed)	.874	
	Total	16		Exact Sig. (2-tailed)	.933	
SMB1	Water (control)	14	0.393	Mann-Whitney U	4	
	Sodium bicarbonate (re-ppt)	2	0.544	Asymp. Sig. (2-tailed)	.112	
	Total	16		Exact Sig. (2-tailed)	.150	
SMB2	Water (control)	14	0.384	Mann-Whitney U	2	
	Sodium bicarbonate (re-ppt)	2	0.550	Asymp. Sig. (2-tailed)	.057	

Peak	Treatment	N	Median	Test statistics		Conclusion
	Total	16		Exact Sig. (2-tailed)	.067	
TYA1	Water (control)	14	1.818	Mann-Whitney U	0	Sodium bicarbonate (re-ppt) = unsafe
	Sodium bicarbonate (re-ppt)	2	1.361	Asymp. Sig. (2-tailed)	.026	
	Total	16		Exact Sig. (2-tailed)	.017	
TYA2	Water (control)	14	1.848	Mann-Whitney U	0	
	Sodium bicarbonate (re-ppt)	2	1.459	Asymp. Sig. (2-tailed)	.026	
	Total	16		Exact Sig. (2-tailed)	.017	
TYB1	Water (control)	14	2.652	Mann-Whitney U	0	
	Sodium bicarbonate (re-ppt)	2	1.660	Asymp. Sig. (2-tailed)	.026	
	Total	16		Exact Sig. (2-tailed)	.017	
TYB2	Water (control)	14	2.669	Mann-Whitney U	0	
	Sodium bicarbonate (re-ppt)	2	1.943	Asymp. Sig. (2-tailed)	.026	
	Total	16		Exact Sig. (2-tailed)	.017	
TYC1	Water (control)	14	2.108	Mann-Whitney U	0	
	Sodium bicarbonate (re-ppt)	2	1.114	Asymp. Sig. (2-tailed)	.026	
	Total	16		Exact Sig. (2-tailed)	.017	
TYC2	Water (control)	14	2.136	Mann-Whitney U	0	
	Sodium bicarbonate (re-ppt)	2	1.178	Asymp. Sig. (2-tailed)	.026	
	Total	16		Exact Sig. (2-tailed)	.017	
TYD2	Water (control)	14	1.832	Mann-Whitney U	0	
	Sodium bicarbonate (re-ppt)	2	0.836	Asymp. Sig. (2-tailed)	.026	
	Total	16		Exact Sig. (2-tailed)	.017	
TYE1	Water (control)	14	4.148	Mann-Whitney U	0	
	Sodium bicarbonate (re-ppt)	2	1.110	Asymp. Sig. (2-tailed)	.026	
	Total	16		Exact Sig. (2-tailed)	.017	
TYE2	Water (control)	14	4.094	Mann-Whitney U	0	
	Sodium bicarbonate (re-ppt)	2	1.181	Asymp. Sig. (2-tailed)	.026	
	Total	16		Exact Sig. (2-tailed)	.017	
SMA1	1:1 ethanol:ether	4	1.020	Mann-Whitney U	7.5	1:1 ethanol:ether = safe compared to ethanol
	Ethanol	4	0.956	Asymp. Sig. (2-tailed)	.885	
	Total	8		Exact Sig. (2-tailed)	.943	
SMA2	1:1 ethanol:ether	4	1.008	Mann-Whitney U	7	
	Ethanol	4	0.963	Asymp. Sig. (2-tailed)	.773	
	Total	8		Exact Sig. (2-tailed)	.886	
SMB1	1:1 ethanol:ether	4	0.444	Mann-Whitney U	8	
	Ethanol	4	0.441	Asymp. Sig. (2-tailed)	1.000	
	Total	8		Exact Sig. (2-tailed)	1.000	
SMB2	1:1 ethanol:ether	4	0.441	Mann-Whitney U	7	
	Ethanol	4	0.419	Asymp. Sig. (2-tailed)	.773	
	Total	8		Exact Sig. (2-tailed)	.886	
TYA1	1:1 ethanol:ether	4	1.701	Mann-Whitney U	6	1:1 ethanol:ether = safe compared to ethanol
	Ethanol	4	1.608	Asymp. Sig. (2-tailed)	.564	
	Total	8		Exact Sig. (2-tailed)	.686	
TYA2	1:1 ethanol:ether	4	1.766	Mann-Whitney U	4	
	Ethanol	4	1.622	Asymp. Sig. (2-tailed)	.248	
	Total	8		Exact Sig. (2-tailed)	.343	
TYB1	1:1 ethanol:ether	4	2.481	Mann-Whitney U	6	
	Ethanol	4	2.343	Asymp. Sig. (2-tailed)	.564	
	Total	8		Exact Sig. (2-tailed)	.686	
TYB2	1:1 ethanol:ether	4	2.518	Mann-Whitney U	3	
	Ethanol	4	2.339	Asymp. Sig. (2-tailed)	.149	
	Total	8		Exact Sig. (2-tailed)	.200	
TYC1	1:1 ethanol:ether	4	2.154	Mann-Whitney U	7	
	Ethanol	4	1.945	Asymp. Sig. (2-tailed)	.773	
	Total	8		Exact Sig. (2-tailed)	.886	
TYC2	1:1 ethanol:ether	4	2.184	Mann-Whitney U	6	
	Ethanol	4	2.059	Asymp. Sig. (2-tailed)	.564	
	Total	8		Exact Sig. (2-tailed)	.686	
TYD1	1:1 ethanol:ether	4	1.849	Mann-Whitney U	5	
	Ethanol	4	1.742	Asymp. Sig. (2-tailed)	.386	
	Total	8		Exact Sig. (2-tailed)	.486	
TYD2	1:1 ethanol:ether	4	1.885	Mann-Whitney U	0	

Peak	Treatment	N	Median	Test statistics		Conclusion
	Ethanol	4	1.792	Asymp. Sig. (2-tailed)	.021	
	Total	8		Exact Sig. (2-tailed)	.029	
TYE1	1:1 ethanol:ether	4	4.023	Mann-Whitney U	1	
	Ethanol	4	3.540	Asymp. Sig. (2-tailed)	.043	
	Total	8		Exact Sig. (2-tailed)	.057	
TYE2	1:1 ethanol:ether	4	4.261	Mann-Whitney U	0	
	Ethanol	4	3.739	Asymp. Sig. (2-tailed)	.021	
	Total	8		Exact Sig. (2-tailed)	.029	
SMA1	1:1 acetone:IMS	4	0.984	Mann-Whitney U	4	
	Acetone	4	0.952	Asymp. Sig. (2-tailed)	.248	
	Total	8		Exact Sig. (2-tailed)	.343	
SMA2	1:1 acetone:IMS	4	1.005	Mann-Whitney U	2	
	Acetone	4	0.914	Asymp. Sig. (2-tailed)	.081	1:1
	Total	8		Exact Sig. (2-tailed)	.114	acetone:IMS =
SMB1	1:1 acetone:IMS	4	0.423	Mann-Whitney U	5.5	comparable to
	Acetone	4	0.433	Asymp. Sig. (2-tailed)	.468	acetone
	Total	8		Exact Sig. (2-tailed)	.571	
SMB2	1:1 acetone:IMS	4	0.434	Mann-Whitney U	3	
	Acetone	4	0.425	Asymp. Sig. (2-tailed)	.149	
	Total	8		Exact Sig. (2-tailed)	.200	
TYA1	1:1 acetone:IMS	4	1.713	Mann-Whitney U	6	
	Acetone	4	1.835	Asymp. Sig. (2-tailed)	.564	
	Total	8		Exact Sig. (2-tailed)	.686	
TYA2	1:1 acetone:IMS	4	1.725	Mann-Whitney U	4	
	Acetone	4	1.839	Asymp. Sig. (2-tailed)	.248	
	Total	8		Exact Sig. (2-tailed)	.343	
TYB1	1:1 acetone:IMS	4	2.406	Mann-Whitney U	4	
	Acetone	4	2.616	Asymp. Sig. (2-tailed)	.248	
	Total	8		Exact Sig. (2-tailed)	.343	
TYB2	1:1 acetone:IMS	4	2.469	Mann-Whitney U	5	
	Acetone	4	2.619	Asymp. Sig. (2-tailed)	.386	
	Total	8		Exact Sig. (2-tailed)	.486	
TYC1	1:1 acetone:IMS	4	2.067	Mann-Whitney U	8	
	Acetone	4	2.229	Asymp. Sig. (2-tailed)	1.000	1:1
	Total	8		Exact Sig. (2-tailed)	1.000	acetone:IMS =
TYC2	1:1 acetone:IMS	4	2.168	Mann-Whitney U	6	comparable to
	Acetone	4	2.264	Asymp. Sig. (2-tailed)	.564	acetone
	Total	8		Exact Sig. (2-tailed)	.686	
TYD1	1:1 acetone:IMS	4	1.926	Mann-Whitney U	7	
	Acetone	4	1.985	Asymp. Sig. (2-tailed)	.773	
	Total	8		Exact Sig. (2-tailed)	.886	
TYD2	1:1 acetone:IMS	4	1.879	Mann-Whitney U	8	
	Acetone	4	1.918	Asymp. Sig. (2-tailed)	1.000	
	Total	8		Exact Sig. (2-tailed)	1.000	
TYE1	1:1 acetone:IMS	4	3.678	Mann-Whitney U	5	
	Acetone	4	3.937	Asymp. Sig. (2-tailed)	.386	
	Total	8		Exact Sig. (2-tailed)	.486	
TYE2	1:1 acetone:IMS	4	3.778	Mann-Whitney U	4.5	
	Acetone	4	3.999	Asymp. Sig. (2-tailed)	.309	
	Total	8		Exact Sig. (2-tailed)	.400	
SMA1	1:1 acetone:IMS	4	0.984	Mann-Whitney U	2	
	IMS	4	0.919	Asymp. Sig. (2-tailed)	.083	
	Total	8		Exact Sig. (2-tailed)	.114	
SMA2	1:1 acetone:IMS	4	1.005	Mann-Whitney U	2	
	IMS	4	0.887	Asymp. Sig. (2-tailed)	.083	1:1
	Total	8		Exact Sig. (2-tailed)	.114	acetone:IMS =
SMB1	1:1 acetone:IMS	4	0.423	Mann-Whitney U	7	comparable to
	IMS	4	0.421	Asymp. Sig. (2-tailed)	.773	IMS
	Total	8		Exact Sig. (2-tailed)	.886	
SMB2	1:1 acetone:IMS	4	0.434	Mann-Whitney U	2	
	IMS	4	0.399	Asymp. Sig. (2-tailed)	.083	
	Total	8		Exact Sig. (2-tailed)	.114	

Peak	Treatment	N	Median	Test statistics		Conclusion
TYA1	1:1 acetone:IMS	4	1.713	Mann-Whitney U	4	
	IMS	4	1.778	Asymp. Sig. (2-tailed)	.248	
	Total	8		Exact Sig. (2-tailed)	.343	
TYA2	1:1 acetone:IMS	4	1.725	Mann-Whitney U	3	
	IMS	4	1.860	Asymp. Sig. (2-tailed)	.149	
	Total	8		Exact Sig. (2-tailed)	.200	
TYB1	1:1 acetone:IMS	4	2.406	Mann-Whitney U	3	
	IMS	4	2.563	Asymp. Sig. (2-tailed)	.146	
	Total	8		Exact Sig. (2-tailed)	.171	
TYB2	1:1 acetone:IMS	4	2.469	Mann-Whitney U	3	
	IMS	4	2.645	Asymp. Sig. (2-tailed)	.149	
	Total	8		Exact Sig. (2-tailed)	.200	
TYC1	1:1 acetone:IMS	4	2.067	Mann-Whitney U	3	
	IMS	4	2.184	Asymp. Sig. (2-tailed)	.149	1:1 acetone:IMS = unsafe compared to IMS
	Total	8		Exact Sig. (2-tailed)	.200	
TYC2	1:1 acetone:IMS	4	2.168	Mann-Whitney U	3	
	IMS	4	2.336	Asymp. Sig. (2-tailed)	.149	
	Total	8		Exact Sig. (2-tailed)	.200	
TYD1	1:1 acetone:IMS	4	1.926	Mann-Whitney U	4	
	IMS	4	2.088	Asymp. Sig. (2-tailed)	.248	
	Total	8		Exact Sig. (2-tailed)	.343	
TYD2	1:1 acetone:IMS	4	1.879	Mann-Whitney U	1	
	IMS	4	2.146	Asymp. Sig. (2-tailed)	.043	
	Total	8		Exact Sig. (2-tailed)	.057	
TYE1	1:1 acetone:IMS	4	3.678	Mann-Whitney U	4	
	IMS	4	4.171	Asymp. Sig. (2-tailed)	.248	
	Total	8		Exact Sig. (2-tailed)	.343	
TYE2	1:1 acetone:IMS	4	3.778	Mann-Whitney U	1	
	IMS	4	4.379	Asymp. Sig. (2-tailed)	.043	
	Total	8		Exact Sig. (2-tailed)	.057	
SMA1	Potassium carbonate	4	1.596	Mann-Whitney U	0	Heating potassium carbonate = unsafe
	Potassium carbonate - 6H @ 80°C	3	0.231	Asymp. Sig. (2-tailed)	.034	
	Total	7		Exact Sig. (2-tailed)	.057	
TYA1	Potassium carbonate	4	1.596	Mann-Whitney U	0	Heating potassium carbonate = unsafe
	Potassium carbonate - 6H @ 80°C	4	0.487	Asymp. Sig. (2-tailed)	.021	
	Total	8		Exact Sig. (2-tailed)	.029	
SMA1	PVAC	4	0.985	Mann-Whitney U	0	
	PVAC/PVAL	4	0.842	Asymp. Sig. (2-tailed)	.021	
	Total	8		Exact Sig. (2-tailed)	.029	
SMA2	PVAC	4	0.934	Mann-Whitney U	1	
	PVAC/PVAL	4	0.850	Asymp. Sig. (2-tailed)	.043	PVAC/PVAL = unsafe compared to PVAC
	Total	8		Exact Sig. (2-tailed)	.057	
SMB1	PVAC	4	0.445	Mann-Whitney U	0	
	PVAC/PVAL	4	0.387	Asymp. Sig. (2-tailed)	.021	
	Total	8		Exact Sig. (2-tailed)	.029	
SMB2	PVAC	4	0.416	Mann-Whitney U	.5	
	PVAC/PVAL	4	0.381	Asymp. Sig. (2-tailed)	.029	
	Total	8		Exact Sig. (2-tailed)	.057	
TYA1	PVAC	4	1.532	Mann-Whitney U	0	PVAC/PVAL = unsafe compared to PVAC
	PVAC/PVAL	4	1.204	Asymp. Sig. (2-tailed)	.021	
	Total	8		Exact Sig. (2-tailed)	.029	
TYA2	PVAC	4	1.551	Mann-Whitney U	2	
	PVAC/PVAL	4	1.322	Asymp. Sig. (2-tailed)	.083	
	Total	8		Exact Sig. (2-tailed)	.114	
TYB1	PVAC	4	2.206	Mann-Whitney U	0	
	PVAC/PVAL	4	1.705	Asymp. Sig. (2-tailed)	.021	
	Total	8		Exact Sig. (2-tailed)	.029	
TYB2	PVAC	4	2.265	Mann-Whitney U	1	
	PVAC/PVAL	4	1.861	Asymp. Sig. (2-tailed)	.043	
	Total	8		Exact Sig. (2-tailed)	.057	
TYC1	PVAC	4	1.780	Mann-Whitney U	0	
	PVAC/PVAL	4	1.422	Asymp. Sig. (2-tailed)	.021	

Peak	Treatment	N	Median	Test statistics		Conclusion
SMA1	Sodium chloride	4	1.078	Mann-Whitney U	0	Sodium sulfide in saline solution = unsafe compared to saline solution
	Sodium sulfide	4	0.095	Asymp. Sig. (2-tailed)	.021	
	Total	8		Exact Sig. (2-tailed)	.029	
SMA2	Sodium chloride	4	1.030	Mann-Whitney U	0	
	Sodium sulfide	4	0.086	Asymp. Sig. (2-tailed)	.021	
	Total	8		Exact Sig. (2-tailed)	.029	
SMB1	Sodium chloride	4	0.489	Mann-Whitney U	0	
	Sodium sulfide	4	0.068	Asymp. Sig. (2-tailed)	.020	
	Total	8		Exact Sig. (2-tailed)	.029	
TYA1	Sodium chloride	4	1.787	Mann-Whitney U	0	Sodium sulfide in saline solution = unsafe compared to saline solution
	Sodium sulfide	4	0.168	Asymp. Sig. (2-tailed)	.021	
	Total	8		Exact Sig. (2-tailed)	.029	
TYA2	Sodium chloride	4	1.891	Mann-Whitney U	0	
	Sodium sulfide	4	0.192	Asymp. Sig. (2-tailed)	.021	
	Total	8		Exact Sig. (2-tailed)	.029	
TYB1	Sodium chloride	4	2.609	Mann-Whitney U	0	
	Sodium sulfide	3	0.278	Asymp. Sig. (2-tailed)	.034	
	Total	7		Exact Sig. (2-tailed)	.057	
TYB2	Sodium chloride	4	2.748	Mann-Whitney U	0	
	Sodium sulfide	4	0.253	Asymp. Sig. (2-tailed)	.021	
	Total	8		Exact Sig. (2-tailed)	.029	
TYC1	Sodium chloride	4	2.101	Mann-Whitney U	0	
	Sodium sulfide	3	0.127	Asymp. Sig. (2-tailed)	.034	
	Total	7		Exact Sig. (2-tailed)	.057	
TYC2	Sodium chloride	4	2.312	Mann-Whitney U	0	
	Sodium sulfide	4	0.122	Asymp. Sig. (2-tailed)	.021	
	Total	8		Exact Sig. (2-tailed)	.029	
TYD1	Sodium chloride	4	1.930	Mann-Whitney U	0	
	Sodium sulfide	3	0.133	Asymp. Sig. (2-tailed)	.034	
	Total	7		Exact Sig. (2-tailed)	.057	
TYD2	Sodium chloride	4	2.098	Mann-Whitney U	0	
	Sodium sulfide	4	0.122	Asymp. Sig. (2-tailed)	.021	
	Total	8		Exact Sig. (2-tailed)	.029	
TYE1	Sodium chloride	4	4.090	Mann-Whitney U	0	
	Sodium sulfide	3	0.209	Asymp. Sig. (2-tailed)	.034	
	Total	7		Exact Sig. (2-tailed)	.057	
TYE2	Sodium chloride	4	4.290	Mann-Whitney U	0	
	Sodium sulfide	4	0.185	Asymp. Sig. (2-tailed)	.021	
	Total	8		Exact Sig. (2-tailed)	.029	
SMA1	Sodium chloride	4	1.078	Mann-Whitney U	0	Sodium sulfide heated in saline solution = unsafe compared to saline solution
	Sodium sulfide - 6H @ 80°C	3	0.090	Asymp. Sig. (2-tailed)	.034	
	Total	7		Exact Sig. (2-tailed)	.057	
SMA2	Sodium chloride	4	1.030	Mann-Whitney U	0	
	Sodium sulfide - 6H @ 80°C	3	0.093	Asymp. Sig. (2-tailed)	.034	
	Total	7		Exact Sig. (2-tailed)	.057	
SMB1	Sodium chloride	4	0.489	Mann-Whitney U	0	
	Sodium sulfide - 6H @ 80°C	3	0.057	Asymp. Sig. (2-tailed)	.034	
	Total	7		Exact Sig. (2-tailed)	.057	
TYA1	Sodium chloride	4	1.787	Mann-Whitney U	0	Sodium sulfide heated in saline solution = unsafe compared to saline solution
	Sodium sulfide - 6H @ 80°C	4	0.370	Asymp. Sig. (2-tailed)	.021	
	Total	8		Exact Sig. (2-tailed)	.029	
TYA2	Sodium chloride	4	1.891	Mann-Whitney U	0	
	Sodium sulfide - 6H @ 80°C	3	0.362	Asymp. Sig. (2-tailed)	.034	
	Total	7		Exact Sig. (2-tailed)	.057	
TYB1	Sodium chloride	4	2.609	Mann-Whitney U	0	
	Sodium sulfide - 6H @ 80°C	3	0.522	Asymp. Sig. (2-tailed)	.034	
	Total	7		Exact Sig. (2-tailed)	.057	
TYB2	Sodium chloride	4	2.748	Mann-Whitney U	0	
	Sodium sulfide - 6H @ 80°C	3	0.503	Asymp. Sig. (2-tailed)	.032	
	Total	7		Exact Sig. (2-tailed)	.057	
TYC1	Sodium chloride	4	2.101	Mann-Whitney U	0	

Peak	Treatment	N	Median	Test statistics		Conclusion
	Sodium sulfide - 6H @ 80°C	3	0.254	Asymp. Sig. (2-tailed)	.034	
	Total	7		Exact Sig. (2-tailed)	.057	
	Sodium chloride	4	2.312	Mann-Whitney U	0	
TYC2	Sodium sulfide - 6H @ 80°C	3	0.253	Asymp. Sig. (2-tailed)	.034	
	Total	7		Exact Sig. (2-tailed)	.057	
	Sodium chloride	4	1.930	Mann-Whitney U	0	
TYD1	Sodium sulfide - 6H @ 80°C	3	0.245	Asymp. Sig. (2-tailed)	.034	
	Total	7		Exact Sig. (2-tailed)	.057	
	Sodium chloride	4	2.098	Mann-Whitney U	0	
TYD2	Sodium sulfide - 6H @ 80°C	3	0.235	Asymp. Sig. (2-tailed)	.034	
	Total	7		Exact Sig. (2-tailed)	.057	
	Sodium chloride	4	4.090	Mann-Whitney U	0	
TYE1	Sodium sulfide - 6H @ 80°C	3	0.369	Asymp. Sig. (2-tailed)	.034	
	Total	7		Exact Sig. (2-tailed)	.057	
	Sodium chloride	4	4.290	Mann-Whitney U	0	
TYE2	Sodium sulfide - 6H @ 80°C	3	0.359	Asymp. Sig. (2-tailed)	.034	
	Total	7		Exact Sig. (2-tailed)	.057	
	Sodium sulfide	4	0.095	Mann-Whitney U	4	
SMA1	Sodium sulfide - 6H @ 80°C	3	0.090	Asymp. Sig. (2-tailed)	.480	
	Total	7		Exact Sig. (2-tailed)	.629	Heating sodium sulfide to 80°C for 6 hours = comparable to sodium sulfide (unheated)
	Sodium sulfide	4	0.086	Mann-Whitney U	4	
SMA2	Sodium sulfide - 6H @ 80°C	3	0.093	Asymp. Sig. (2-tailed)	.480	
	Total	7		Exact Sig. (2-tailed)	.629	
	Sodium sulfide	4	0.068	Mann-Whitney U	4	
SMB1	Sodium sulfide - 6H @ 80°C	3	0.057	Asymp. Sig. (2-tailed)	.476	
	Total	7		Exact Sig. (2-tailed)	.543	
	Sodium sulfide	4	0.168	Mann-Whitney U	4	
TYA1	Sodium sulfide - 6H @ 80°C	4	0.370	Asymp. Sig. (2-tailed)	.248	
	Total	8		Exact Sig. (2-tailed)	.343	
	Sodium sulfide	4	0.192	Mann-Whitney U	0	
TYA2	Sodium sulfide - 6H @ 80°C	3	0.362	Asymp. Sig. (2-tailed)	.034	
	Total	7		Exact Sig. (2-tailed)	.057	
	Sodium sulfide	4	0.253	Mann-Whitney U	0	Heating sodium sulfide to 80°C for 6 hours = safe compared to sodium sulfide (unheated)
TYB2	Sodium sulfide - 6H @ 80°C	3	0.503	Asymp. Sig. (2-tailed)	.032	
	Total	7		Exact Sig. (2-tailed)	.057	
	Sodium sulfide	4	0.122	Mann-Whitney U	0	
TYC2	Sodium sulfide - 6H @ 80°C	3	0.253	Asymp. Sig. (2-tailed)	.034	
	Total	7		Exact Sig. (2-tailed)	.057	
	Sodium sulfide	4	0.122	Mann-Whitney U	3	
TYD2	Sodium sulfide - 6H @ 80°C	3	0.235	Asymp. Sig. (2-tailed)	.289	
	Total	7		Exact Sig. (2-tailed)	.400	
	Sodium sulfide	4	0.185	Mann-Whitney U	3	
TYE2	Sodium sulfide - 6H @ 80°C	3	0.359	Asymp. Sig. (2-tailed)	.289	
	Total	7		Exact Sig. (2-tailed)	.400	
	1:1 ethanol:ether	4	1.020	Mann-Whitney U	8	
SMA1	Cellulose nitrate	4	0.901	Asymp. Sig. (2-tailed)	1.000	
	Total	8		Exact Sig. (2-tailed)	1.000	
	1:1 ethanol:ether	4	1.008	Mann-Whitney U	7	
SMA2	Cellulose nitrate	4	0.875	Asymp. Sig. (2-tailed)	.773	Cellulose nitrate (in 1:1 ethanol:ether) = comparable to 1:1 ethanol: ether
	Total	8		Exact Sig. (2-tailed)	.886	
	1:1 ethanol:ether	4	0.444	Mann-Whitney U	6	
SMB1	Cellulose nitrate	4	0.408	Asymp. Sig. (2-tailed)	.564	
	Total	8		Exact Sig. (2-tailed)	.686	
	1:1 ethanol:ether	4	0.441	Mann-Whitney U	7	
SMB2	Cellulose nitrate	4	0.377	Asymp. Sig. (2-tailed)	.773	
	Total	8		Exact Sig. (2-tailed)	.886	
	1:1 ethanol:ether	4	1.701	Mann-Whitney U	5	
TYA1	Cellulose nitrate	4	1.460	Asymp. Sig. (2-tailed)	.386	Cellulose nitrate (in 1:1 ethanol:ether) = unsafe compared to 1:1 ethanol: ether
	Total	8		Exact Sig. (2-tailed)	.486	
	1:1 ethanol:ether	4	1.766	Mann-Whitney U	4	
TYA2	Cellulose nitrate	4	1.421	Asymp. Sig. (2-tailed)	.245	
	Total	8		Exact Sig. (2-tailed)	.314	

Peak	Treatment	N	Median	Test statistics		Conclusion
TYB1	1:1 ethanol:ether	4	2.481	Mann-Whitney U	5	
	Cellulose nitrate	4	2.192	Asymp. Sig. (2-tailed)	.386	
	Total	8		Exact Sig. (2-tailed)	.486	
TYB2	1:1 ethanol:ether	4	2.518	Mann-Whitney U	4	
	Cellulose nitrate	4	2.057	Asymp. Sig. (2-tailed)	.248	
	Total	8		Exact Sig. (2-tailed)	.343	
TYC1	1:1 ethanol:ether	4	2.154	Mann-Whitney U	5	
	Cellulose nitrate	4	1.822	Asymp. Sig. (2-tailed)	.386	
	Total	8		Exact Sig. (2-tailed)	.486	
TYC2	1:1 ethanol:ether	4	2.184	Mann-Whitney U	4	
	Cellulose nitrate	4	1.711	Asymp. Sig. (2-tailed)	.248	
	Total	8		Exact Sig. (2-tailed)	.343	
TYD1	1:1 ethanol:ether	4	1.849	Mann-Whitney U	7	
	Cellulose nitrate	4	1.780	Asymp. Sig. (2-tailed)	.773	
	Total	8		Exact Sig. (2-tailed)	.886	
TYD2	1:1 ethanol:ether	4	1.885	Mann-Whitney U	3	
	Cellulose nitrate	4	1.674	Asymp. Sig. (2-tailed)	.149	
	Total	8		Exact Sig. (2-tailed)	.200	
TYE1	1:1 ethanol:ether	4	4.023	Mann-Whitney U	5	
	Cellulose nitrate	4	3.641	Asymp. Sig. (2-tailed)	.386	
	Total	8		Exact Sig. (2-tailed)	.486	
TYE2	1:1 ethanol:ether	4	4.261	Mann-Whitney U	1	
	Cellulose nitrate	4	3.484	Asymp. Sig. (2-tailed)	.043	
	Total	8		Exact Sig. (2-tailed)	.057	
SMA1	Cellulose nitrate	4	0.901	Mann-Whitney U	6	
	Ethanol	4	0.956	Asymp. Sig. (2-tailed)	.564	
	Total	8		Exact Sig. (2-tailed)	.686	
SMA2	Cellulose nitrate	4	0.875	Mann-Whitney U	4	
	Ethanol	4	0.963	Asymp. Sig. (2-tailed)	.248	Cellulose nitrate (in 1:1 ethanol:ether) = comparable to ethanol
	Total	8		Exact Sig. (2-tailed)	.343	
SMB1	Cellulose nitrate	4	0.408	Mann-Whitney U	5	
	Ethanol	4	0.441	Asymp. Sig. (2-tailed)	.386	
	Total	8		Exact Sig. (2-tailed)	.486	
SMB2	Cellulose nitrate	4	0.377	Mann-Whitney U	4	
	Ethanol	4	0.419	Asymp. Sig. (2-tailed)	.248	
	Total	8		Exact Sig. (2-tailed)	.343	
TYA1	Cellulose nitrate	4	1.460	Mann-Whitney U	4.5	Cellulose nitrate (in 1:1 ethanol:ether) = comparable to ethanol
	Ethanol	4	1.608	Asymp. Sig. (2-tailed)	.309	
	Total	8		Exact Sig. (2-tailed)	.371	
TYA2	Cellulose nitrate	4	1.421	Mann-Whitney U	4	
	Ethanol	4	1.622	Asymp. Sig. (2-tailed)	.245	
	Total	8		Exact Sig. (2-tailed)	.314	
TYB1	Cellulose nitrate	4	2.192	Mann-Whitney U	5	
	Ethanol	4	2.343	Asymp. Sig. (2-tailed)	.386	
	Total	8		Exact Sig. (2-tailed)	.486	
TYB2	Cellulose nitrate	4	2.057	Mann-Whitney U	4	
	Ethanol	4	2.339	Asymp. Sig. (2-tailed)	.248	
	Total	8		Exact Sig. (2-tailed)	.343	
TYC1	Cellulose nitrate	4	1.822	Mann-Whitney U	6	
	Ethanol	4	1.945	Asymp. Sig. (2-tailed)	.564	
	Total	8		Exact Sig. (2-tailed)	.686	
TYC2	Cellulose nitrate	4	1.711	Mann-Whitney U	4	
	Ethanol	4	2.059	Asymp. Sig. (2-tailed)	.248	
	Total	8		Exact Sig. (2-tailed)	.343	
TYD1	Cellulose nitrate	4	1.780	Mann-Whitney U	7	
	Ethanol	4	1.742	Asymp. Sig. (2-tailed)	.773	
	Total	8		Exact Sig. (2-tailed)	.886	
TYD2	Cellulose nitrate	4	1.674	Mann-Whitney U	4	
	Ethanol	4	1.792	Asymp. Sig. (2-tailed)	.248	
	Total	8		Exact Sig. (2-tailed)	.343	
TYE1	Cellulose nitrate	4	3.641	Mann-Whitney U	7	
	Ethanol	4	3.540	Asymp. Sig. (2-tailed)	.773	

Peak	Treatment	N	Median	Test statistics		Conclusion
	Total	8		Exact Sig. (2-tailed)	.886	
	Cellulose nitrate	4	3.484	Mann-Whitney U	4	
TYE2	Ethanol	4	3.739	Asymp. Sig. (2-tailed)	.248	
	Total	8		Exact Sig. (2-tailed)	.343	
	Ethanol	4	0.956	Mann-Whitney U	0	
SMA1	Gasoline	4	0.672	Asymp. Sig. (2-tailed)	.021	
	Total	8		Exact Sig. (2-tailed)	.029	
	Ethanol	4	0.963	Mann-Whitney U	0	
SMA2	Gasoline	4	0.656	Asymp. Sig. (2-tailed)	.021	Gasoline = unsafe compared to ethanol
	Total	8		Exact Sig. (2-tailed)	.029	
	Ethanol	4	0.441	Mann-Whitney U	0	
SMB1	Gasoline	4	0.312	Asymp. Sig. (2-tailed)	.021	
	Total	8		Exact Sig. (2-tailed)	.029	
	Ethanol	4	0.419	Mann-Whitney U	0	
SMB2	Gasoline	4	0.294	Asymp. Sig. (2-tailed)	.021	
	Total	8		Exact Sig. (2-tailed)	.029	
	Ethanol	4	1.608	Mann-Whitney U	0	
TYA1	Gasoline	4	1.414	Asymp. Sig. (2-tailed)	.021	
	Total	8		Exact Sig. (2-tailed)	.029	
	Ethanol	4	1.622	Mann-Whitney U	0	
TYA2	Gasoline	4	1.440	Asymp. Sig. (2-tailed)	.021	
	Total	8		Exact Sig. (2-tailed)	.029	
	Ethanol	4	2.343	Mann-Whitney U	0	
TYB1	Gasoline	4	2.028	Asymp. Sig. (2-tailed)	.021	
	Total	8		Exact Sig. (2-tailed)	.029	
	Ethanol	4	2.339	Mann-Whitney U	0	
TYB2	Gasoline	4	2.060	Asymp. Sig. (2-tailed)	.020	
	Total	8		Exact Sig. (2-tailed)	.029	
	Ethanol	4	1.945	Mann-Whitney U	0	
TYC1	Gasoline	4	1.712	Asymp. Sig. (2-tailed)	.021	Gasoline = unsafe compared to ethanol
	Total	8		Exact Sig. (2-tailed)	.029	
	Ethanol	4	2.059	Mann-Whitney U	0	
TYC2	Gasoline	4	1.732	Asymp. Sig. (2-tailed)	.021	
	Total	8		Exact Sig. (2-tailed)	.029	
	Ethanol	4	1.742	Mann-Whitney U	4	
TYD1	Gasoline	4	1.642	Asymp. Sig. (2-tailed)	.248	
	Total	8		Exact Sig. (2-tailed)	.343	
	Ethanol	4	1.792	Mann-Whitney U	1	
TYD2	Gasoline	4	1.653	Asymp. Sig. (2-tailed)	.043	
	Total	8		Exact Sig. (2-tailed)	.057	
	Ethanol	4	3.540	Mann-Whitney U	6	
TYE1	Gasoline	4	3.373	Asymp. Sig. (2-tailed)	.564	
	Total	8		Exact Sig. (2-tailed)	.686	
	Ethanol	4	3.739	Mann-Whitney U	5	
TYE2	Gasoline	4	3.503	Asymp. Sig. (2-tailed)	.386	
	Total	8		Exact Sig. (2-tailed)	.486	
	Gasoline	4	1.414	Mann-Whitney U	0	Turpentine = unsafe compared to gasoline
TYA1	Turpentine	4	0.217	Asymp. Sig. (2-tailed)	.021	
	Total	8		Exact Sig. (2-tailed)	.029	
	Gasoline	4	1.440	Mann-Whitney U	0	
TYA2	Turpentine	4	0.220	Asymp. Sig. (2-tailed)	.021	
	Total	8		Exact Sig. (2-tailed)	.029	
	Gasoline	4	2.028	Mann-Whitney U	0	
TYB1	Turpentine	4	0.321	Asymp. Sig. (2-tailed)	.021	
	Total	8		Exact Sig. (2-tailed)	.029	
	Gasoline	4	2.060	Mann-Whitney U	0	
TYB2	Turpentine	4	0.319	Asymp. Sig. (2-tailed)	.020	
	Total	8		Exact Sig. (2-tailed)	.029	
	Gasoline	4	1.712	Mann-Whitney U	0	
TYC1	Turpentine	4	0.149	Asymp. Sig. (2-tailed)	.021	
	Total	8		Exact Sig. (2-tailed)	.029	
TYC2	Gasoline	4	1.732	Mann-Whitney U	0	

Peak	Treatment	N	Median	Test statistics		Conclusion
	Turpentine	4	0.131	Asymp. Sig. (2-tailed)	.021	
	Total	8		Exact Sig. (2-tailed)	.029	
TYD1	Gasoline	4	1.642	Mann-Whitney U	0	
	Turpentine	4	0.138	Asymp. Sig. (2-tailed)	.021	
	Total	8		Exact Sig. (2-tailed)	.029	
TYD2	Gasoline	4	1.653	Mann-Whitney U	0	
	Turpentine	4	0.125	Asymp. Sig. (2-tailed)	.021	
	Total	8		Exact Sig. (2-tailed)	.029	
TYE1	Gasoline	4	3.373	Mann-Whitney U	0	
	Turpentine	4	0.241	Asymp. Sig. (2-tailed)	.021	
	Total	8		Exact Sig. (2-tailed)	.029	
TYE2	Gasoline	4	3.503	Mann-Whitney U	0	
	Turpentine	4	0.209	Asymp. Sig. (2-tailed)	.021	
	Total	8		Exact Sig. (2-tailed)	.029	
TYA1	Linseed oil	4	1.467	Mann-Whitney U	0	
	Turpentine	4	0.217	Asymp. Sig. (2-tailed)	.021	
	Total	8		Exact Sig. (2-tailed)	.029	
TYA2	Linseed oil	4	1.512	Mann-Whitney U	0	
	Turpentine	4	0.220	Asymp. Sig. (2-tailed)	.021	
	Total	8		Exact Sig. (2-tailed)	.029	
TYB1	Linseed oil	4	2.222	Mann-Whitney U	0	
	Turpentine	4	0.321	Asymp. Sig. (2-tailed)	.021	
	Total	8		Exact Sig. (2-tailed)	.029	
TYB2	Linseed oil	4	2.196	Mann-Whitney U	0	
	Turpentine	4	0.319	Asymp. Sig. (2-tailed)	.021	
	Total	8		Exact Sig. (2-tailed)	.029	
TYC1	Linseed oil	4	1.768	Mann-Whitney U	0	
	Turpentine	4	0.149	Asymp. Sig. (2-tailed)	.021	Turpentine = unsafe compared to linseed oil
	Total	8		Exact Sig. (2-tailed)	.029	
TYC2	Linseed oil	4	1.815	Mann-Whitney U	0	
	Turpentine	4	0.131	Asymp. Sig. (2-tailed)	.021	
	Total	8		Exact Sig. (2-tailed)	.029	
TYD1	Linseed oil	4	1.709	Mann-Whitney U	0	
	Turpentine	4	0.138	Asymp. Sig. (2-tailed)	.021	
	Total	8		Exact Sig. (2-tailed)	.029	
TYD2	Linseed oil	4	1.694	Mann-Whitney U	0	
	Turpentine	4	0.125	Asymp. Sig. (2-tailed)	.021	
	Total	8		Exact Sig. (2-tailed)	.029	
TYE1	Linseed oil	4	4.429	Mann-Whitney U	0	
	Turpentine	4	0.241	Asymp. Sig. (2-tailed)	.021	
	Total	8		Exact Sig. (2-tailed)	.029	
TYE2	Linseed oil	4	4.412	Mann-Whitney U	0	
	Turpentine	4	0.209	Asymp. Sig. (2-tailed)	.021	
	Total	8		Exact Sig. (2-tailed)	.029	
SMA1	Acetone	4	0.952	Mann-Whitney U	4	
	Methylmethacrylate/ethylacrylate	4	0.830	Asymp. Sig. (2-tailed)	.248	
	Total	8		Exact Sig. (2-tailed)	.343	
SMA2	Acetone	4	0.914	Mann-Whitney U	4	Methyl-methacrylate/ ethylacrylate (in acetone) = comparable to acetone
	Methylmethacrylate/ethylacrylate	4	0.829	Asymp. Sig. (2-tailed)	.245	
	Total	8		Exact Sig. (2-tailed)	.286	
SMB1	Acetone	4	0.433	Mann-Whitney U	3	
	Methylmethacrylate/ethylacrylate	4	0.364	Asymp. Sig. (2-tailed)	.149	
	Total	8		Exact Sig. (2-tailed)	.200	
SMB2	Acetone	4	0.425	Mann-Whitney U	3.5	
	Methylmethacrylate/ethylacrylate	4	0.359	Asymp. Sig. (2-tailed)	.191	
	Total	8		Exact Sig. (2-tailed)	.229	
TYA1	Acetone	4	1.835	Mann-Whitney U	4	Methyl-methacrylate/ ethacrylate (in acetone) = unsafe compared to acetone
	Methylmethacrylate/ethylacrylate	4	1.616	Asymp. Sig. (2-tailed)	.248	
	Total	8		Exact Sig. (2-tailed)	.343	
TYA2	Acetone	4	1.839	Mann-Whitney U	5	
	Methylmethacrylate/ethylacrylate	4	1.621	Asymp. Sig. (2-tailed)	.386	
	Total	8		Exact Sig. (2-tailed)	.486	

Peak	Treatment	N	Median	Test statistics		Conclusion
TYB1	Acetone	4	2.616	Mann-Whitney U	4	
	Methylmethacrylate/ethylacrylate	4	2.352	Asymp. Sig. (2-tailed)	.248	
	Total	8		Exact Sig. (2-tailed)	.343	
TYB2	Acetone	4	2.619	Mann-Whitney U	5	
	Methylmethacrylate/ethylacrylate	4	2.352	Asymp. Sig. (2-tailed)	.386	
	Total	8		Exact Sig. (2-tailed)	.486	
TYC1	Acetone	4	2.229	Mann-Whitney U	7	
	Methylmethacrylate/ethylacrylate	4	2.032	Asymp. Sig. (2-tailed)	.773	
	Total	8		Exact Sig. (2-tailed)	.886	
TYC2	Acetone	4	2.264	Mann-Whitney U	6	
	Methylmethacrylate/ethylacrylate	4	2.024	Asymp. Sig. (2-tailed)	.564	
	Total	8		Exact Sig. (2-tailed)	.686	
TYD1	Acetone	4	1.985	Mann-Whitney U	4	
	Methylmethacrylate/ethylacrylate	4	1.747	Asymp. Sig. (2-tailed)	.248	
	Total	8		Exact Sig. (2-tailed)	.343	
TYD2	Acetone	4	1.918	Mann-Whitney U	3	
	Methylmethacrylate/ethylacrylate	4	1.716	Asymp. Sig. (2-tailed)	.149	
	Total	8		Exact Sig. (2-tailed)	.200	
TYE1	Acetone	4	3.937	Mann-Whitney U	0	
	Methylmethacrylate/ethylacrylate	4	3.358	Asymp. Sig. (2-tailed)	.021	
	Total	8		Exact Sig. (2-tailed)	.029	
TYE2	Acetone	4	3.999	Mann-Whitney U	1	
	Methylmethacrylate/ethylacrylate	4	3.298	Asymp. Sig. (2-tailed)	.043	
	Total	8		Exact Sig. (2-tailed)	.057	
SMA1	1:1 acetone:IMS	4	0.984	Mann-Whitney U	4	
	Poly(vinyl) butyral resin	4	0.947	Asymp. Sig. (2-tailed)	.248	
	Total	8		Exact Sig. (2-tailed)	.343	
SMA2	1:1 acetone:IMS	4	1.005	Mann-Whitney U	4	Poly(vinyl) butyral resin (in 1:1 acetone:IMS) = comparable to 1:1 acetone:IMS
	Poly(vinyl) butyral resin	4	0.915	Asymp. Sig. (2-tailed)	.248	
	Total	8		Exact Sig. (2-tailed)	.343	
SMB1	1:1 acetone:IMS	4	0.423	Mann-Whitney U	6	
	Poly(vinyl) butyral resin	4	0.479	Asymp. Sig. (2-tailed)	.564	
	Total	8		Exact Sig. (2-tailed)	.686	
SMB2	1:1 acetone:IMS	4	0.434	Mann-Whitney U	5.5	
	Poly(vinyl) butyral resin	4	0.421	Asymp. Sig. (2-tailed)	.468	
	Total	8		Exact Sig. (2-tailed)	.571	
TYA1	1:1 acetone:IMS	4	1.713	Mann-Whitney U	0	Poly(vinyl) butyral resin (in 1:1 acetone:IMS) = unsafe compared to 1:1 acetone:IMS
	Poly(vinyl) butyral resin	4	1.383	Asymp. Sig. (2-tailed)	.021	
	Total	8		Exact Sig. (2-tailed)	.029	
TYA2	1:1 acetone:IMS	4	1.725	Mann-Whitney U	0	
	Poly(vinyl) butyral resin	4	1.486	Asymp. Sig. (2-tailed)	.021	
	Total	8		Exact Sig. (2-tailed)	.029	
TYB1	1:1 acetone:IMS	4	2.406	Mann-Whitney U	2	
	Poly(vinyl) butyral resin	4	1.929	Asymp. Sig. (2-tailed)	.083	
	Total	8		Exact Sig. (2-tailed)	.114	
TYB2	1:1 acetone:IMS	4	2.469	Mann-Whitney U	2	
	Poly(vinyl) butyral resin	4	2.079	Asymp. Sig. (2-tailed)	.083	
	Total	8		Exact Sig. (2-tailed)	.114	
TYC1	1:1 acetone:IMS	4	2.067	Mann-Whitney U	2	
	Poly(vinyl) butyral resin	4	1.661	Asymp. Sig. (2-tailed)	.083	
	Total	8		Exact Sig. (2-tailed)	.114	
TYC2	1:1 acetone:IMS	4	2.168	Mann-Whitney U	0	
	Poly(vinyl) butyral resin	4	1.710	Asymp. Sig. (2-tailed)	.021	
	Total	8		Exact Sig. (2-tailed)	.029	
TYD1	1:1 acetone:IMS	4	1.926	Mann-Whitney U	2	
	Poly(vinyl) butyral resin	4	1.595	Asymp. Sig. (2-tailed)	.083	
	Total	8		Exact Sig. (2-tailed)	.114	
TYD2	1:1 acetone:IMS	4	1.879	Mann-Whitney U	2	
	Poly(vinyl) butyral resin	4	1.654	Asymp. Sig. (2-tailed)	.083	
	Total	8		Exact Sig. (2-tailed)	.114	
TYE1	1:1 acetone:IMS	4	3.678	Mann-Whitney U	1	
	Poly(vinyl) butyral resin	4	2.912	Asymp. Sig. (2-tailed)	.043	

Peak	Treatment	N	Median	Test statistics		Conclusion
	Total	8		Exact Sig. (2-tailed)	.057	
TYE2	1:1 acetone:IMS	4	3.778	Mann-Whitney U	1	
	Poly(vinyl) butyral resin	4	3.267	Asymp. Sig. (2-tailed)	.043	
	Total	8		Exact Sig. (2-tailed)	.057	
SMA1	Acetone	4	0.952	Mann-Whitney U	7	Poly(vinyl) butyral resin (in 1:1 acetone:IMS) = comparable to acetone
	Poly(vinyl) butyral resin	4	0.947	Asymp. Sig. (2-tailed)	.773	
	Total	8		Exact Sig. (2-tailed)	.886	
SMA2	Acetone	4	0.914	Mann-Whitney U	7	
	Poly(vinyl) butyral resin	4	0.915	Asymp. Sig. (2-tailed)	.772	
	Total	8		Exact Sig. (2-tailed)	.800	
SMB1	Acetone	4	0.433	Mann-Whitney U	5	
	Poly(vinyl) butyral resin	4	0.479	Asymp. Sig. (2-tailed)	.386	
	Total	8		Exact Sig. (2-tailed)	.486	
SMB2	Acetone	4	0.425	Mann-Whitney U	8	
	Poly(vinyl) butyral resin	4	0.421	Asymp. Sig. (2-tailed)	1.000	
	Total	8		Exact Sig. (2-tailed)	1.000	
TYA1	Acetone	4	1.835	Mann-Whitney U	0	Poly(vinyl) butyral resin (in 1:1 acetone:IMS) = unsafe compared to acetone
	Poly(vinyl) butyral resin	4	1.383	Asymp. Sig. (2-tailed)	.021	
	Total	8		Exact Sig. (2-tailed)	.029	
TYA2	Acetone	4	1.839	Mann-Whitney U	0	
	Poly(vinyl) butyral resin	4	1.486	Asymp. Sig. (2-tailed)	.021	
	Total	8		Exact Sig. (2-tailed)	.029	
TYB1	Acetone	4	2.616	Mann-Whitney U	0	
	Poly(vinyl) butyral resin	4	1.929	Asymp. Sig. (2-tailed)	.021	
	Total	8		Exact Sig. (2-tailed)	.029	
TYB2	Acetone	4	2.619	Mann-Whitney U	0	
	Poly(vinyl) butyral resin	4	2.079	Asymp. Sig. (2-tailed)	.021	
	Total	8		Exact Sig. (2-tailed)	.029	
TYC1	Acetone	4	2.229	Mann-Whitney U	2	
	Poly(vinyl) butyral resin	4	1.661	Asymp. Sig. (2-tailed)	.083	
	Total	8		Exact Sig. (2-tailed)	.114	
TYC2	Acetone	4	2.264	Mann-Whitney U	0	
	Poly(vinyl) butyral resin	4	1.710	Asymp. Sig. (2-tailed)	.021	
	Total	8		Exact Sig. (2-tailed)	.029	
TYD1	Acetone	4	1.985	Mann-Whitney U	1	
	Poly(vinyl) butyral resin	4	1.595	Asymp. Sig. (2-tailed)	.043	
	Total	8		Exact Sig. (2-tailed)	.057	
TYD2	Acetone	4	1.918	Mann-Whitney U	2	
	Poly(vinyl) butyral resin	4	1.654	Asymp. Sig. (2-tailed)	.083	
	Total	8		Exact Sig. (2-tailed)	.114	
TYE1	Acetone	4	3.937	Mann-Whitney U	0	
	Poly(vinyl) butyral resin	4	2.912	Asymp. Sig. (2-tailed)	.021	
	Total	8		Exact Sig. (2-tailed)	.029	
TYE2	Acetone	4	3.999	Mann-Whitney U	1	
	Poly(vinyl) butyral resin	4	3.267	Asymp. Sig. (2-tailed)	.043	
	Total	8		Exact Sig. (2-tailed)	.057	
SMA1	IMS	4	0.919	Mann-Whitney U	6	Poly(vinyl) butyral resin (in 1:1 acetone:IMS) = comparable to IMS
	Poly(vinyl) butyral resin	4	0.947	Asymp. Sig. (2-tailed)	.564	
	Total	8		Exact Sig. (2-tailed)	.686	
SMA2	IMS	4	0.887	Mann-Whitney U	5	
	Poly(vinyl) butyral resin	4	0.915	Asymp. Sig. (2-tailed)	.386	
	Total	8		Exact Sig. (2-tailed)	.486	
SMB1	IMS	4	0.421	Mann-Whitney U	4	
	Poly(vinyl) butyral resin	4	0.479	Asymp. Sig. (2-tailed)	.248	
	Total	8		Exact Sig. (2-tailed)	.343	
SMB2	IMS	4	0.399	Mann-Whitney U	4	
	Poly(vinyl) butyral resin	4	0.421	Asymp. Sig. (2-tailed)	.248	
	Total	8		Exact Sig. (2-tailed)	.343	

Peak	Treatment	N	Median	Test statistics		Conclusion
TYA1	IMS	4	1.778	Mann-Whitney U	0	
	Poly(vinyl) butyral resin	4	1.383	Asymp. Sig. (2-tailed)	.021	
	Total	8		Exact Sig. (2-tailed)	.029	
TYA2	IMS	4	1.860	Mann-Whitney U	0	
	Poly(vinyl) butyral resin	4	1.486	Asymp. Sig. (2-tailed)	.021	
	Total	8		Exact Sig. (2-tailed)	.029	
TYB1	IMS	4	2.563	Mann-Whitney U	0	
	Poly(vinyl) butyral resin	4	1.929	Asymp. Sig. (2-tailed)	.020	
	Total	8		Exact Sig. (2-tailed)	.029	
TYB2	IMS	4	2.645	Mann-Whitney U	0	
	Poly(vinyl) butyral resin	4	2.079	Asymp. Sig. (2-tailed)	.021	
	Total	8		Exact Sig. (2-tailed)	.029	
TYC1	IMS	4	2.184	Mann-Whitney U	0	Poly(vinyl) butyral resin (in 1:1 acetone:IMS) = unsafe compared to IMS
	Poly(vinyl) butyral resin	4	1.661	Asymp. Sig. (2-tailed)	.021	
	Total	8		Exact Sig. (2-tailed)	.029	
TYC2	IMS	4	2.336	Mann-Whitney U	0	
	Poly(vinyl) butyral resin	4	1.710	Asymp. Sig. (2-tailed)	.021	
	Total	8		Exact Sig. (2-tailed)	.029	
TYD1	IMS	4	2.088	Mann-Whitney U	0	
	Poly(vinyl) butyral resin	4	1.595	Asymp. Sig. (2-tailed)	.021	
	Total	8		Exact Sig. (2-tailed)	.029	
TYD2	IMS	4	2.146	Mann-Whitney U	0	
	Poly(vinyl) butyral resin	4	1.654	Asymp. Sig. (2-tailed)	.021	
	Total	8		Exact Sig. (2-tailed)	.029	
TYE1	IMS	4	4.171	Mann-Whitney U	0	
	Poly(vinyl) butyral resin	4	2.912	Asymp. Sig. (2-tailed)	.021	
	Total	8		Exact Sig. (2-tailed)	.029	
TYE2	IMS	4	4.379	Mann-Whitney U	0	
	Poly(vinyl) butyral resin	4	3.267	Asymp. Sig. (2-tailed)	.021	
	Total	8		Exact Sig. (2-tailed)	.029	
SMA1	Water (control) - ether extracted (2)	6	0.865	Mann-Whitney U	10	
	Water (control) (2)	4	0.854	Asymp. Sig. (2-tailed)	.670	
	Total	10		Exact Sig. (2-tailed)	.762	
SMA2	Water (control) - ether extracted (2)	6	0.838	Mann-Whitney U	10	
	Water (control) (2)	4	0.832	Asymp. Sig. (2-tailed)	.670	
	Total	10		Exact Sig. (2-tailed)	.762	Water – ether extracted = safe
SMB1	Water (control) - ether extracted (2)	6	0.472	Mann-Whitney U	9	
	Water (control) (2)	4	0.461	Asymp. Sig. (2-tailed)	.522	
	Total	10		Exact Sig. (2-tailed)	.610	
SMB2	Water (control) - ether extracted (2)	6	0.459	Mann-Whitney U	9	
	Water (control) (2)	4	0.454	Asymp. Sig. (2-tailed)	.522	
	Total	10		Exact Sig. (2-tailed)	.610	
TYA1	Water (control) - ether extracted (2)	6	1.571	Mann-Whitney U	9.5	Water – ether extracted = safe
	Water (control) (2)	4	1.545	Asymp. Sig. (2-tailed)	.593	
	Total	10		Exact Sig. (2-tailed)	.657	
TYA2	Water (control) - ether extracted (2)	6	1.612	Mann-Whitney U	10	
	Water (control) (2)	4	1.594	Asymp. Sig. (2-tailed)	.669	
	Total	10		Exact Sig. (2-tailed)	.710	
TYB1	Water (control) - ether extracted (2)	6	2.201	Mann-Whitney U	10	
	Water (control) (2)	4	2.249	Asymp. Sig. (2-tailed)	.670	
	Total	10		Exact Sig. (2-tailed)	.762	
TYB2	Water (control) - ether extracted (2)	6	2.250	Mann-Whitney U	12	
	Water (control) (2)	4	2.277	Asymp. Sig. (2-tailed)	1.000	
	Total	10		Exact Sig. (2-tailed)	1.000	
TYC1	Water (control) - ether extracted (2)	6	1.115	Mann-Whitney U	5	
	Water (control) (2)	4	1.056	Asymp. Sig. (2-tailed)	.136	
	Total	10		Exact Sig. (2-tailed)	.171	
TYC2	Water (control) - ether extracted (2)	6	1.137	Mann-Whitney U	8	
	Water (control) (2)	4	1.077	Asymp. Sig. (2-tailed)	.394	
	Total	10		Exact Sig. (2-tailed)	.476	
TYD1	Water (control) - ether extracted (2)	6	1.035	Mann-Whitney U	11	

Peak	Treatment	N	Median	Test statistics		Conclusion
	Water (control) (2)	4	1.055	Asymp. Sig. (2-tailed)	.831	
	Total	10		Exact Sig. (2-tailed)	.914	
TYD2	Water (control) - ether extracted (2)	6	1.062	Mann-Whitney U	12	
	Water (control) (2)	4	1.056	Asymp. Sig. (2-tailed)	1.000	
	Total	10		Exact Sig. (2-tailed)	1.000	
TYE1	Water (control) - ether extracted (2)	6	1.535	Mann-Whitney U	10	
	Water (control) (2)	4	1.587	Asymp. Sig. (2-tailed)	.670	
	Total	10		Exact Sig. (2-tailed)	.762	
TYE2	Water (control) - ether extracted (2)	6	1.623	Mann-Whitney U	12	
	Water (control) (2)	4	1.642	Asymp. Sig. (2-tailed)	1.000	
	Total	10		Exact Sig. (2-tailed)	1.000	
SMA1	Water (control) (2)	4	0.854	Mann-Whitney U	8	Heating water to 37°C for 48 hours = unsafe
	Water (control) - 48H @ 37°C (2)	4	0.854	Asymp. Sig. (2-tailed)	1.000	
	Total	8		Exact Sig. (2-tailed)	1.000	
SMA2	Water (control) (2)	4	0.832	Mann-Whitney U	6	
	Water (control) - 48H @ 37°C (2)	4	0.791	Asymp. Sig. (2-tailed)	.564	
	Total	8		Exact Sig. (2-tailed)	.686	
SMB1	Water (control) (2)	4	0.461	Mann-Whitney U	7	
	Water (control) - 48H @ 37°C (2)	4	0.464	Asymp. Sig. (2-tailed)	.773	
	Total	8		Exact Sig. (2-tailed)	.886	
SMB2	Water (control) (2)	4	0.454	Mann-Whitney U	0	
	Water (control) - 48H @ 37°C (2)	4	0.335	Asymp. Sig. (2-tailed)	.021	
	Total	8		Exact Sig. (2-tailed)	.029	
TYA1	Water (control) (2)	4	1.545	Mann-Whitney U	8	Heating water to 37°C for 48 hours = safe
	Water (control) - 48H @ 37°C (2)	4	1.558	Asymp. Sig. (2-tailed)	1.000	
	Total	8		Exact Sig. (2-tailed)	1.000	
TYA2	Water (control) (2)	4	1.594	Mann-Whitney U	8	
	Water (control) - 48H @ 37°C (2)	4	1.595	Asymp. Sig. (2-tailed)	1.000	
	Total	8		Exact Sig. (2-tailed)	1.000	
TYB1	Water (control) (2)	4	2.249	Mann-Whitney U	7	
	Water (control) - 48H @ 37°C (2)	4	2.232	Asymp. Sig. (2-tailed)	.773	
	Total	8		Exact Sig. (2-tailed)	.886	
TYB2	Water (control) (2)	4	2.277	Mann-Whitney U	8	
	Water (control) - 48H @ 37°C (2)	4	2.287	Asymp. Sig. (2-tailed)	1.000	
	Total	8		Exact Sig. (2-tailed)	1.000	
TYC1	Water (control) (2)	4	1.056	Mann-Whitney U	8	
	Water (control) - 48H @ 37°C (2)	4	1.072	Asymp. Sig. (2-tailed)	1.000	
	Total	8		Exact Sig. (2-tailed)	1.000	
TYC2	Water (control) (2)	4	1.077	Mann-Whitney U	0	
	Water (control) - 48H @ 37°C (2)	4	1.853	Asymp. Sig. (2-tailed)	.021	
	Total	8		Exact Sig. (2-tailed)	.029	
TYD1	Water (control) (2)	4	1.055	Mann-Whitney U	7	
	Water (control) - 48H @ 37°C (2)	4	1.030	Asymp. Sig. (2-tailed)	.773	
	Total	8		Exact Sig. (2-tailed)	.886	
TYD2	Water (control) (2)	4	1.056	Mann-Whitney U	4	
	Water (control) - 48H @ 37°C (2)	4	1.263	Asymp. Sig. (2-tailed)	.248	
	Total	8		Exact Sig. (2-tailed)	.343	
TYE1	Water (control) (2)	4	1.587	Mann-Whitney U	7	
	Water (control) - 48H @ 37°C (2)	4	1.555	Asymp. Sig. (2-tailed)	.773	
	Total	8		Exact Sig. (2-tailed)	.886	
TYE2	Water (control) (2)	4	1.642	Mann-Whitney U	0	
	Water (control) - 48H @ 37°C (2)	4	3.454	Asymp. Sig. (2-tailed)	.021	
	Total	8		Exact Sig. (2-tailed)	.029	
SMA1	Water (control) (2)	4	0.854	Mann-Whitney U	0	Heating water to 100°C and allowing to cool to room temperature overnight = unsafe
	Water (control) - O/N from 100°C to RT (2)	4	0.398	Asymp. Sig. (2-tailed)	.021	
	Total	8		Exact Sig. (2-tailed)	.029	
SMA2	Water (control) (2)	4	0.832	Mann-Whitney U	0	
	Water (control) - O/N from 100°C to RT (2)	4	0.380	Asymp. Sig. (2-tailed)	.021	
	Total	8		Exact Sig. (2-tailed)	.029	
SMB1	Water (control) (2)	4	0.461	Mann-Whitney U	0	
	Water (control) - O/N from 100°C to RT (2)	4	0.214	Asymp. Sig. (2-tailed)	.021	
	Total	8		Exact Sig. (2-tailed)	.029	

Peak	Treatment	N	Median	Test statistics		Conclusion
SMB2	Water (control) (2)	4	0.454	Mann-Whitney U	0	
	Water (control) - O/N from 100°C to RT (2)	3	0.204	Asymp. Sig. (2-tailed)	.034	
	Total	7		Exact Sig. (2-tailed)	.057	
TYA1	Water (control) (2)	4	1.545	Mann-Whitney U	0	Heating water to 100°C and allowing to cool to room temperature overnight = unsafe
	Water (control) - O/N from 100°C to RT (2)	4	1.289	Asymp. Sig. (2-tailed)	.021	
	Total	8		Exact Sig. (2-tailed)	.029	
TYA2	Water (control) (2)	4	1.594	Mann-Whitney U	0	
	Water (control) - O/N from 100°C to RT (2)	4	1.185	Asymp. Sig. (2-tailed)	.021	
	Total	8		Exact Sig. (2-tailed)	.029	
TYB1	Water (control) (2)	4	2.249	Mann-Whitney U	0	
	Water (control) - O/N from 100°C to RT (2)	4	1.837	Asymp. Sig. (2-tailed)	.021	
	Total	8		Exact Sig. (2-tailed)	.029	
TYB2	Water (control) (2)	4	2.277	Mann-Whitney U	0	
	Water (control) - O/N from 100°C to RT (2)	4	1.633	Asymp. Sig. (2-tailed)	.021	
	Total	8		Exact Sig. (2-tailed)	.029	
TYC1	Water (control) (2)	4	1.056	Mann-Whitney U	0	
	Water (control) - O/N from 100°C to RT (2)	4	0.899	Asymp. Sig. (2-tailed)	.021	
	Total	8		Exact Sig. (2-tailed)	.029	
TYC2	Water (control) (2)	4	1.077	Mann-Whitney U	0	
	Water (control) - O/N from 100°C to RT (2)	4	0.863	Asymp. Sig. (2-tailed)	.021	
	Total	8		Exact Sig. (2-tailed)	.029	
TYD1	Water (control) (2)	4	1.055	Mann-Whitney U	0	
	Water (control) - O/N from 100°C to RT (2)	4	0.774	Asymp. Sig. (2-tailed)	.021	
	Total	8		Exact Sig. (2-tailed)	.029	
TYD2	Water (control) (2)	4	1.056	Mann-Whitney U	0	
	Water (control) - O/N from 100°C to RT (2)	4	0.729	Asymp. Sig. (2-tailed)	.021	
	Total	8		Exact Sig. (2-tailed)	.029	
TYE1	Water (control) (2)	4	1.587	Mann-Whitney U	0	
	Water (control) - O/N from 100°C to RT (2)	4	1.155	Asymp. Sig. (2-tailed)	.021	
	Total	8		Exact Sig. (2-tailed)	.029	
TYE2	Water (control) (2)	4	1.642	Mann-Whitney U	0	
	Water (control) - O/N from 100°C to RT (2)	4	1.113	Asymp. Sig. (2-tailed)	.021	
	Total	8		Exact Sig. (2-tailed)	.029	
SMA1	Water (control) (2)	4	0.854	Mann-Whitney U	4	Not heating or mixing = safe
	Water (control) - unheated, unmixed (2)	3	0.926	Asymp. Sig. (2-tailed)	.480	
	Total	7		Exact Sig. (2-tailed)	.629	
SMA2	Water (control) (2)	4	0.832	Mann-Whitney U	1	
	Water (control) - unheated, unmixed (2)	3	0.939	Asymp. Sig. (2-tailed)	.077	
	Total	7		Exact Sig. (2-tailed)	.114	
SMB1	Water (control) (2)	4	0.461	Mann-Whitney U	4	
	Water (control) - unheated, unmixed (2)	3	0.508	Asymp. Sig. (2-tailed)	.480	
	Total	7		Exact Sig. (2-tailed)	.629	
SMB2	Water (control) (2)	4	0.454	Mann-Whitney U	.5	
	Water (control) - unheated, unmixed (2)	3	0.508	Asymp. Sig. (2-tailed)	.050	
	Total	7		Exact Sig. (2-tailed)	.086	
TYA1	Water (control) (2)	4	1.545	Mann-Whitney U	0	Not heating or mixing = mixed result
	Water (control) - unheated, unmixed (2)	3	1.720	Asymp. Sig. (2-tailed)	.034	
	Total	7		Exact Sig. (2-tailed)	.057	
TYA2	Water (control) (2)	4	1.594	Mann-Whitney U	0	
	Water (control) - unheated, unmixed (2)	3	1.825	Asymp. Sig. (2-tailed)	.034	
	Total	7		Exact Sig. (2-tailed)	.057	
TYB1	Water (control) (2)	4	2.249	Mann-Whitney U	1	
	Water (control) - unheated, unmixed (2)	3	2.358	Asymp. Sig. (2-tailed)	.077	
	Total	7		Exact Sig. (2-tailed)	.114	
TYB2	Water (control) (2)	4	2.277	Mann-Whitney U	0	
	Water (control) - unheated, unmixed (2)	3	2.625	Asymp. Sig. (2-tailed)	.034	
	Total	7		Exact Sig. (2-tailed)	.057	
TYC1	Water (control) (2)	4	1.056	Mann-Whitney U	0	
	Water (control) - unheated, unmixed (2)	3	1.223	Asymp. Sig. (2-tailed)	.034	
	Total	7		Exact Sig. (2-tailed)	.057	
TYC2	Water (control) (2)	4	1.077	Mann-Whitney U	0	
	Water (control) - unheated, unmixed (2)	3	1.213	Asymp. Sig. (2-tailed)	.034	

Peak	Treatment	N	Median	Test statistics		Conclusion
	Total	7		Exact Sig. (2-tailed)	.057	
TYD1	Water (control) (2)	4	1.055	Mann-Whitney U	4	
	Water (control) - unheated, unmixed (2)	3	1.099	Asymp. Sig. (2-tailed)	.480	
	Total	7		Exact Sig. (2-tailed)	.629	
TYD2	Water (control) (2)	4	1.056	Mann-Whitney U	0	
	Water (control) - unheated, unmixed (2)	3	0.967	Asymp. Sig. (2-tailed)	.034	
	Total	7		Exact Sig. (2-tailed)	.057	
TYE1	Water (control) (2)	4	1.587	Mann-Whitney U	5	
	Water (control) - unheated, unmixed (2)	3	1.626	Asymp. Sig. (2-tailed)	.724	
	Total	7		Exact Sig. (2-tailed)	.857	
TYE2	Water (control) (2)	4	1.642	Mann-Whitney U	2	
	Water (control) - unheated, unmixed (2)	3	1.815	Asymp. Sig. (2-tailed)	.157	
	Total	7		Exact Sig. (2-tailed)	.229	
TYA1	Water (control) (2)	4	1.545	Mann-Whitney U	0	Detergent = unsafe
	Detergent (2)	3	1.067	Asymp. Sig. (2-tailed)	.034	
	Total	7		Exact Sig. (2-tailed)	.057	
TYB1	Water (control) (2)	4	2.249	Mann-Whitney U	0	
	Detergent (2)	3	1.280	Asymp. Sig. (2-tailed)	.034	
	Total	7		Exact Sig. (2-tailed)	.057	
TYC1	Water (control) (2)	4	1.056	Mann-Whitney U	0	
	Detergent (2)	3	0.795	Asymp. Sig. (2-tailed)	.034	
	Total	7		Exact Sig. (2-tailed)	.057	
SMA1	Water (control) (2)	4	0.854	Mann-Whitney U	0	Mercury (II) chloride = unsafe
	Mercury (II) chloride (2)	4	0.290	Asymp. Sig. (2-tailed)	.021	
	Total	8		Exact Sig. (2-tailed)	.029	
SMA2	Water (control) (2)	4	0.832	Mann-Whitney U	0	
	Mercury (II) chloride (2)	4	0.303	Asymp. Sig. (2-tailed)	.021	
	Total	8		Exact Sig. (2-tailed)	.029	
SMB1	Water (control) (2)	4	0.461	Mann-Whitney U	0	
	Mercury (II) chloride (2)	4	0.185	Asymp. Sig. (2-tailed)	.021	
	Total	8		Exact Sig. (2-tailed)	.029	
SMB2	Water (control) (2)	4	0.454	Mann-Whitney U	0	
	Mercury (II) chloride (2)	4	0.192	Asymp. Sig. (2-tailed)	.021	
	Total	8		Exact Sig. (2-tailed)	.029	
TYA1	Water (control) (2)	4	1.545	Mann-Whitney U	0	Mercury (II) chloride = unsafe
	Mercury (II) chloride (2)	4	0.753	Asymp. Sig. (2-tailed)	.021	
	Total	8		Exact Sig. (2-tailed)	.029	
TYA2	Water (control) (2)	4	1.594	Mann-Whitney U	0	
	Mercury (II) chloride (2)	4	0.742	Asymp. Sig. (2-tailed)	.021	
	Total	8		Exact Sig. (2-tailed)	.029	
TYB1	Water (control) (2)	4	2.249	Mann-Whitney U	0	
	Mercury (II) chloride (2)	4	1.037	Asymp. Sig. (2-tailed)	.021	
	Total	8		Exact Sig. (2-tailed)	.029	
TYB2	Water (control) (2)	4	2.277	Mann-Whitney U	0	
	Mercury (II) chloride (2)	4	1.033	Asymp. Sig. (2-tailed)	.021	
	Total	8		Exact Sig. (2-tailed)	.029	
TYC1	Water (control) (2)	4	1.056	Mann-Whitney U	0	
	Mercury (II) chloride (2)	4	0.469	Asymp. Sig. (2-tailed)	.021	
	Total	8		Exact Sig. (2-tailed)	.029	
TYC2	Water (control) (2)	4	1.077	Mann-Whitney U	0	
	Mercury (II) chloride (2)	4	0.462	Asymp. Sig. (2-tailed)	.021	
	Total	8		Exact Sig. (2-tailed)	.029	
TYD1	Water (control) (2)	4	1.055	Mann-Whitney U	0	
	Mercury (II) chloride (2)	4	0.464	Asymp. Sig. (2-tailed)	.021	
	Total	8		Exact Sig. (2-tailed)	.029	
TYD2	Water (control) (2)	4	1.056	Mann-Whitney U	0	
	Mercury (II) chloride (2)	4	0.446	Asymp. Sig. (2-tailed)	.021	
	Total	8		Exact Sig. (2-tailed)	.029	
TYE1	Water (control) (2)	4	1.587	Mann-Whitney U	0	
	Mercury (II) chloride (2)	4	0.689	Asymp. Sig. (2-tailed)	.021	
	Total	8		Exact Sig. (2-tailed)	.029	
TYE2	Water (control) (2)	4	1.642	Mann-Whitney U	0	

Peak	Treatment	N	Median	Test statistics		Conclusion
	Mercury (II) chloride (2)	4	0.682	Asymp. Sig. (2-tailed)	.021	
	Total	8		Exact Sig. (2-tailed)	.029	
SMA1	Water (control) (2)	4	0.854	Mann-Whitney U	0	Pepsin = unsafe
	Pepsin (2)	4	0.223	Asymp. Sig. (2-tailed)	.021	
	Total	8		Exact Sig. (2-tailed)	.029	
SMA2	Water (control) (2)	4	0.832	Mann-Whitney U	0	
	Pepsin (2)	4	0.280	Asymp. Sig. (2-tailed)	.021	
	Total	8		Exact Sig. (2-tailed)	.029	
SMB1	Water (control) (2)	4	0.461	Mann-Whitney U	0	
	Pepsin (2)	3	0.140	Asymp. Sig. (2-tailed)	.034	
	Total	7		Exact Sig. (2-tailed)	.057	
TYA1	Water (control) (2)	4	1.545	Mann-Whitney U	0	
	Pepsin (2)	4	0.701	Asymp. Sig. (2-tailed)	.021	
	Total	8		Exact Sig. (2-tailed)	.029	
TYA2	Water (control) (2)	4	1.594	Mann-Whitney U	0	
	Pepsin (2)	4	0.579	Asymp. Sig. (2-tailed)	.021	
	Total	8		Exact Sig. (2-tailed)	.029	
TYB1	Water (control) (2)	4	2.249	Mann-Whitney U	0	
	Pepsin (2)	4	0.973	Asymp. Sig. (2-tailed)	.021	
	Total	8		Exact Sig. (2-tailed)	.029	
TYB2	Water (control) (2)	4	2.277	Mann-Whitney U	0	
	Pepsin (2)	4	0.855	Asymp. Sig. (2-tailed)	.021	
	Total	8		Exact Sig. (2-tailed)	.029	
TYC1	Water (control) (2)	4	1.056	Mann-Whitney U	0	
	Pepsin (2)	4	0.481	Asymp. Sig. (2-tailed)	.021	
	Total	8		Exact Sig. (2-tailed)	.029	
TYC2	Water (control) (2)	4	1.077	Mann-Whitney U	0	
	Pepsin (2)	4	0.673	Asymp. Sig. (2-tailed)	.021	
	Total	8		Exact Sig. (2-tailed)	.029	
TYD1	Water (control) (2)	4	1.055	Mann-Whitney U	0	
	Pepsin (2)	4	0.468	Asymp. Sig. (2-tailed)	.021	
	Total	8		Exact Sig. (2-tailed)	.029	
TYD2	Water (control) (2)	4	1.056	Mann-Whitney U	0	
	Pepsin (2)	4	0.697	Asymp. Sig. (2-tailed)	.021	
	Total	8		Exact Sig. (2-tailed)	.029	
TYE1	Water (control) (2)	4	1.587	Mann-Whitney U	0	
	Pepsin (2)	4	0.655	Asymp. Sig. (2-tailed)	.021	
	Total	8		Exact Sig. (2-tailed)	.029	
TYE2	Water (control) (2)	4	1.642	Mann-Whitney U	0	
	Pepsin (2)	4	1.242	Asymp. Sig. (2-tailed)	.021	
	Total	8		Exact Sig. (2-tailed)	.029	
TYA1	Water (control) (2)	4	1.545	Mann-Whitney U	0	Pepsin heated to 37°C for 48 hours = unsafe
	Pepsin - 48H @ 37°C (2)	4	0.186	Asymp. Sig. (2-tailed)	.021	
	Total	8		Exact Sig. (2-tailed)	.029	
TYA2	Water (control) (2)	4	1.594	Mann-Whitney U	0	
	Pepsin - 48H @ 37°C (2)	4	0.208	Asymp. Sig. (2-tailed)	.021	
	Total	8		Exact Sig. (2-tailed)	.029	
TYB1	Water (control) (2)	4	2.249	Mann-Whitney U	0	
	Pepsin - 48H @ 37°C (2)	4	0.252	Asymp. Sig. (2-tailed)	.021	
	Total	8		Exact Sig. (2-tailed)	.029	
TYB2	Water (control) (2)	4	2.277	Mann-Whitney U	0	
	Pepsin - 48H @ 37°C (2)	4	0.292	Asymp. Sig. (2-tailed)	.021	
	Total	8		Exact Sig. (2-tailed)	.029	
TYC1	Water (control) (2)	4	1.056	Mann-Whitney U	0	
	Pepsin - 48H @ 37°C (2)	4	0.128	Asymp. Sig. (2-tailed)	.021	
	Total	8		Exact Sig. (2-tailed)	.029	
TYC2	Water (control) (2)	4	1.077	Mann-Whitney U	0	
	Pepsin - 48H @ 37°C (2)	4	0.206	Asymp. Sig. (2-tailed)	.021	
	Total	8		Exact Sig. (2-tailed)	.029	
TYD1	Water (control) (2)	4	1.055	Mann-Whitney U	0	
	Pepsin - 48H @ 37°C (2)	4	0.125	Asymp. Sig. (2-tailed)	.021	
	Total	8		Exact Sig. (2-tailed)	.029	

Peak	Treatment	N	Median	Test statistics		Conclusion
TYD2	Water (control) (2)	4	1.056	Mann-Whitney U	0	
	Pepsin - 48H @ 37°C (2)	4	0.154	Asymp. Sig. (2-tailed)	.021	
	Total	8		Exact Sig. (2-tailed)	.029	
TYE1	Water (control) (2)	4	1.587	Mann-Whitney U	0	
	Pepsin - 48H @ 37°C (2)	4	0.188	Asymp. Sig. (2-tailed)	.021	
	Total	8		Exact Sig. (2-tailed)	.029	
TYE2	Water (control) (2)	4	1.642	Mann-Whitney U	0	
	Pepsin - 48H @ 37°C (2)	4	0.447	Asymp. Sig. (2-tailed)	.021	
	Total	8		Exact Sig. (2-tailed)	.029	
TYA1	Water (control) (2)	4	1.545	Mann-Whitney U	0	Sodium bicarb. 24H @ 80°C = unsafe
	Sodium bicarbonate - 24H @ 80°C (2)	3	0.886	Asymp. Sig. (2-tailed)	.034	
	Total	7		Exact Sig. (2-tailed)	.057	
TYA1	Pepsin (2)	4	0.701	Mann-Whitney U	0	
	Pepsin - 48H @ 37°C (2)	4	0.186	Asymp. Sig. (2-tailed)	.021	
	Total	8		Exact Sig. (2-tailed)	.029	
TYA2	Pepsin (2)	4	0.579	Mann-Whitney U	0	
	Pepsin - 48H @ 37°C (2)	4	0.208	Asymp. Sig. (2-tailed)	.021	
	Total	8		Exact Sig. (2-tailed)	.029	
TYB1	Pepsin (2)	4	0.973	Mann-Whitney U	0	
	Pepsin - 48H @ 37°C (2)	4	0.252	Asymp. Sig. (2-tailed)	.021	
	Total	8		Exact Sig. (2-tailed)	.029	
TYB2	Pepsin (2)	4	0.855	Mann-Whitney U	0	
	Pepsin - 48H @ 37°C (2)	4	0.292	Asymp. Sig. (2-tailed)	.021	
	Total	8		Exact Sig. (2-tailed)	.029	
TYC1	Pepsin (2)	4	0.481	Mann-Whitney U	0	Pepsin heated to 37°C for 48 hours = unsafe compared to pepsin (unheated)
	Pepsin - 48H @ 37°C (2)	4	0.128	Asymp. Sig. (2-tailed)	.021	
	Total	8		Exact Sig. (2-tailed)	.029	
TYC2	Pepsin (2)	4	0.673	Mann-Whitney U	0	
	Pepsin - 48H @ 37°C (2)	4	0.206	Asymp. Sig. (2-tailed)	.021	
	Total	8		Exact Sig. (2-tailed)	.029	
TYD1	Pepsin (2)	4	0.468	Mann-Whitney U	0	
	Pepsin - 48H @ 37°C (2)	4	0.125	Asymp. Sig. (2-tailed)	.021	
	Total	8		Exact Sig. (2-tailed)	.029	
TYD2	Pepsin (2)	4	0.697	Mann-Whitney U	0	
	Pepsin - 48H @ 37°C (2)	4	0.154	Asymp. Sig. (2-tailed)	.021	
	Total	8		Exact Sig. (2-tailed)	.029	
TYE1	Pepsin (2)	4	0.655	Mann-Whitney U	0	
	Pepsin - 48H @ 37°C (2)	4	0.188	Asymp. Sig. (2-tailed)	.021	
	Total	8		Exact Sig. (2-tailed)	.029	
TYE2	Pepsin (2)	4	1.242	Mann-Whitney U	0	
	Pepsin - 48H @ 37°C (2)	4	0.447	Asymp. Sig. (2-tailed)	.021	
	Total	8		Exact Sig. (2-tailed)	.029	
TYA1	Water (control) - 48H @ 37°C (2)	4	1.558	Mann-Whitney U	0	Heating pepsin to 37°C for 48 hours = unsafe compared to water heated to 37°C for 48 hours
	Pepsin - 48H @ 37°C (2)	4	0.186	Asymp. Sig. (2-tailed)	.021	
	Total	8		Exact Sig. (2-tailed)	.029	
TYA2	Water (control) - 48H @ 37°C (2)	4	1.595	Mann-Whitney U	0	
	Pepsin - 48H @ 37°C (2)	4	0.208	Asymp. Sig. (2-tailed)	.021	
	Total	8		Exact Sig. (2-tailed)	.029	
TYB1	Water (control) - 48H @ 37°C (2)	4	2.232	Mann-Whitney U	0	
	Pepsin - 48H @ 37°C (2)	4	0.252	Asymp. Sig. (2-tailed)	.021	
	Total	8		Exact Sig. (2-tailed)	.029	
TYB2	Water (control) - 48H @ 37°C (2)	4	2.287	Mann-Whitney U	0	
	Pepsin - 48H @ 37°C (2)	4	0.292	Asymp. Sig. (2-tailed)	.021	
	Total	8		Exact Sig. (2-tailed)	.029	
TYC1	Water (control) - 48H @ 37°C (2)	4	1.072	Mann-Whitney U	0	
	Pepsin - 48H @ 37°C (2)	4	0.128	Asymp. Sig. (2-tailed)	.021	
	Total	8		Exact Sig. (2-tailed)	.029	
TYC2	Water (control) - 48H @ 37°C (2)	4	1.853	Mann-Whitney U	0	
	Pepsin - 48H @ 37°C (2)	4	0.206	Asymp. Sig. (2-tailed)	.021	
	Total	8		Exact Sig. (2-tailed)	.029	
TYD1	Water (control) - 48H @ 37°C (2)	4	1.030	Mann-Whitney U	0	
	Pepsin - 48H @ 37°C (2)	4	0.125	Asymp. Sig. (2-tailed)	.021	

Peak	Treatment	N	Median	Test statistics		Conclusion
	Total	8		Exact Sig. (2-tailed)	.029	
TYD2	Water (control) - 48H @ 37°C (2)	4	1.263	Mann-Whitney U	0	
	Pepsin - 48H @ 37°C (2)	4	0.154	Asymp. Sig. (2-tailed)	.021	
	Total	8		Exact Sig. (2-tailed)	.029	
TYE1	Water (control) - 48H @ 37°C (2)	4	1.555	Mann-Whitney U	0	
	Pepsin - 48H @ 37°C (2)	4	0.188	Asymp. Sig. (2-tailed)	.021	
	Total	8		Exact Sig. (2-tailed)	.029	
TYE2	Water (control) - 48H @ 37°C (2)	4	3.454	Mann-Whitney U	0	
	Pepsin - 48H @ 37°C (2)	4	0.447	Asymp. Sig. (2-tailed)	.021	
	Total	8		Exact Sig. (2-tailed)	.029	